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POSTER PRESENTATIONS

P01 – Apoptosis

P01-1
Expression and activation of the apoptosome apparatus core components in non-small cell lung carcinoma
NSCLC cells

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Formation of functional apoptosome apparatus (AA) is a key event in the course of the mitochondrial mechanism of apoptosis. Downregulated expression, impaired assembly and/or insufficient activity of AA may significantly contribute to tumorigenesis, progression and therapy resistance of neoplasms. We investigated whether DNA methylation is involved in regulation of the transcriptional expression of core AA components, i.e. Apaf-1 and procaspase-9 (PC-9), and the downstream AA effector, i.e. procaspase-3 (PC-3) in NSCLC cells. Moreover, we analysed the functionality of AA in cell-free cytosol from NSCLC cell lines. The treatment with 5-aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, led to significant upregulation of Apaf-1, PC-9 and PC-3 mRNAs expression in 3, 4 and 5 of 7 tested cell lines, respectively. Assays of the (cytochrome-c (cyt-c) + dATP)-induced caspase-3-like activity and of the PC-9 processing to p35 caspase-9 subunit showed that AA was activated in five of seven tested cell lines. The activatability of AA in cell-free cytosols was rapidly lost by preincubation of the samples at 37°C. This was accompanied by formation of high-M_r Apaf-1 aggregates, as demonstrated by gel filtration chromatography and immunoblotting. On the contrary, in non-preincubated cytosols, Apaf-1 eluted in low-M_r fractions, and in the cytosols preincubated with cyt-c and dATP at 37°C, a significant quantity of Apaf-1 eluted in the high-M_r fractions while PC-9 was completely converted to caspase-9 (p35 subunit), which co-eluted with Apaf-1 in the high-M_r fractions and appeared also as a low-M_r free form. In conclusion, our results indicate that DNA methylation can inhibit transcriptional expression of Apaf-1, PC-9 and PC-3 in NSCLC cells. This may increase the apoptotic threshold of the cells. Further, our results demonstrate that AA functionality is impaired in cell-free cytosol from a subset of NSCLC cell lines and is lost by thermal pretreatment of cell-free cytosols due to Apaf-1 aggregation.

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P01-2
Role of ICAM3 and its interacting partners in the phagocytosis of apoptotic neutrophils by macrophages

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Apoptotic cells express eat-me signals which are recognized by several receptors mainly on professional phagocytes that can define their safe and effective clearance in order to maintain tissue homeostasis in the entire body. TLDA measurements predicted important role of some apopto-phagocytic genes in phagocytosis of apoptotic neutrophils by macrophages, because their expression level heavily elevated during the early stage of phagocytosis. After they were silenced by RNA interference, significant decrease in phagocytosis was observed only after silencing ICAM3. Our goal was to investigate the role and interacting partners of ICAM3 from both sides. Monocytes were isolated from buffy coats of healthy blood by CD14 specific magnetic separation. To examine phagocytic capacity of differentiated macrophages, apoptotic neutrophils were isolated from human blood by density-gradient centrifugation. Phagocytosis assay was performed using fluorescent labeled cells and the incorporated cell-rate was measured by flow cytometry, immediately after preincubation of macrophages or apoptotic cells with blocking antibodies. Localization of the investigated receptors was visualized by indirect immunostaining. Significant reduction of phagocytosis was noticed after blocking of ICAM3 from both sides. In macrophages but not in neutrophils silencing and blocking components of LFA-1, which can strongly bind ICAM3, resulted in a decreased phagocytosis of apoptotic cells. Engulfing caps formed in macrophages during phagocytosis are characterized by accumulation of ICAM3 and the subunits of LFA-1 which show co-localization on the surface of phagocytes showing that ICAM3 and LFA-1 act as recognition receptors in the phagocytosis portals of macrophages for engulfment of apoptotic neutrophils.

P01r-3
TMBIM3/GRINA is a novel unfolded protein response (UPR) target gene that controls apoptosis through the modulation of ER calcium homeostasis

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Transmembrane BAX inhibitor motif-containing (TMBIM)-6, also known as BAX-inhibitor 1 (BI-1), is an anti-apoptotic protein that belongs to a putative family of highly conserved and poorly characterized genes. Here we report the function of TMBIM3/GRINA in the control of cell death by endoplasmic reticulum (ER) stress. Tmbim3 mRNA levels are strongly upregulated in cellular and animal models of ER stress, controlled by the PERK signaling branch of the unfolded protein response. TMBIM3/GRINA synergies with TMBIM6/BI-1 in the modulation of ER calcium homeostasis and apoptosis, associated with physical interactions with inositol trisphosphate receptors, which could explain its anti-apoptotic activity under conditions of ER stress. Loss-of-function studies in *D. melanogaster* demonstrated that TMBIM3/GRINA and TMBIM6/BI-1 have synergistic activities against ER stress *in vivo*. Similarly, manipulation of TMBIM3/GRINA levels in zebrafish embryos revealed an essential role in the control of apoptosis during neuronal development and in experimental models of ER stress. These findings suggest the existence of a conserved group of functionally related cell

death regulators across species beyond the BCL-2 family of proteins operating at the ER membrane.

P01-4
The influence of photodynamic reaction in combination with electroporation on human melanotic melanoma cells

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Background: The photodynamic therapy (PDT) is the method of selective tumor treatment. It is based on the specific photosensitizer accumulation in the tumor tissue, followed by irradiation with visible light. Numerous studies suggest that the exposure of tumor cells to PDT can lead to cellular and molecular mechanisms, which mediate oxidative stress in cells. The electroporation (EP) is widely used in biology and medicine. The application of the cell membrane EP in combination with photosensitizers could increase their transport into cells.
Objectives: The aim of this study was to examine an effect of combining both anticancer methods applied *in vitro*. Photodynamic reaction enhanced by electroporation was tested on the human melanotic melanoma cells (MeWo).
Methods: The Photofrin® was used for the photodynamic reaction as a photosensitizer. The cells were incubated for 18 hour with 20 μg/ml of Ph in DMEM. Than they were irradiated 10 min with the light intensity of 10 mW/cm^2 using a lamp with polarized light and red filter (632.8 nm). The electroporation parameters were: 1000 V/cm, 100 μs, eight impulses. The cloning efficacy test was used to determine cells viability. Alkaline and neutral comet assay was used to investigate type of cell death.
Results: It was observed that photodynamic reaction in combination with electroporation is more cytotoxic than photodynamic reaction without electroporation. Comet assay demonstrated much higher percentage of apoptotic cells after photodynamic reaction than necrotic cells, but the highest number of apoptotic cells was observed after EP-PDT.
Conclusions: The experiments proved that electroporation effectively supports photodynamic method. Photodynamic reaction based Ph in combination with EP induced apoptosis in MeWo cells.
Acknowledgments: This work was supported by a grant of the Polish Ministry of Science and Higher Education, grant No. 5409/B/P01/2011/40 (J. Saczko) and grant for Young Scientists sponsored by Statutory Funds of the Wroclaw Medical University, grant No. PBmn1 (J. Saczko).

P01r-5
Visualizing interactions between Bcl-2 proteins in living cells by fluorescence complementation

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The key event in the mitochondrial pathway of apoptosis is the activation of Bax and Bak by BH3-only proteins, through a molecular mechanism that is still a matter of debate. We have studied interactions among anti- and pro-apoptotic proteins of the Bcl-2 family in living cells by using Bimolecular Fluorescence Complementation (BiFC) analysis. Our results indicate that Mcl-1 preferably binds to the BH3-only proteins Bim, Puma and Noxa, but also to Bak and Bax. We have also found a direct interaction between Bim, Puma or Noxa with Bax or Bak during apoptosis induction. Interaction of Bim with Bax occurs in cytosol and then Bim/Bax complexes translocate to mitochondria. Complexes of either Puma or Noxa with Bax or Bak during apoptosis were always detected at mitochondria. Overexpression of Bcl-xL or Mcl-1 delayed Bim/Bax translocation to mitochondria as well as oligomerization of Bax and Bak. Bak oligomerization was strictly dependent on an intact BH3-domain but oligomerization of Bax depended on H1a helix. These results reveal the ability of main BH3-only proteins to directly activate Bax and Bak in living cells and suggest that a complex network of interactions regulate the function of Bcl-2 family members during apoptosis.

P01-6
Combinations with natural and synthetic tea catechins as apoptotic inducers in breast cancer

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We have recently shown that the ester-bonded gallate catechins isolated from green tea, epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG), are potent inhibitors of dihydrofolate reductase (DHFR) activity *in vitro* at concentrations found in the serum and tissues of green tea drinkers. Since this first report describing the antifolate activity of tea polyphenols, several studies by us and other laboratories have confirmed this activity and reported that EGCG inhibits DHFR from a variety of biological sources. Recently, a screening of DHFR-binding drugs by MALDI-TOFMS demonstrated that EGCG is an active inhibitor of DHFR and has a relative affinity between that of pyrimethamine and methotrexate. However, the excellent anticancer properties of tea catechins are significantly limited by their poor bioavailability, which is related to their low stability in neutral or slightly alkaline solutions and their inability to easily cross cellular membranes. In an attempt to solve these bioavailability problems, we synthesised a 3,4,5-trimethoxybenzoyl analogue of catechin gallate (TMCG) that exhibited high antiproliferative activity against epithelial cancer cell lines, including those from breast cancer. Here, we observed that combination of natural or synthetic tea catechins (EGCG or TMCG) with compounds that disrupt adenosine metabolism in cells, such as dipyridamole induced consistent apoptosis in breast cancer cells. This study demonstrates that simultaneous targeting of DNA and protein methylation is an effective epigenetic therapy, which reactivates the expression of pro-apoptotic RASSF1A and induces apoptosis in breast cancer cells.
Acknowledgments: This work was supported by grants from Ministerio de Ciencia e Innovación (MICINN) (SAF2009-12043-C02-01), Fundación Séneca, Región de Murcia (FS-RM) (15230/PI/10) and European Commission (FP7-INCO 293514). M.F.M is contracted by Fundación de la Asociación Española contra el Cáncer (FAECC).

P01-7
Role of autophagy during the development of the vertebrate nervous system

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Autophagy is an evolutionarily conserved process that occurs at basal conditions and promotes cellular homeostasis through the recycling of cellular components and supplying energy during periods of metabolic stress. Additionally, autophagy can lead to rapid cellular changes necessary for proper differentiation and/or development. In this work we studied the role of autophagy in the development of the nervous system. We used as a model the mouse retina in conditions where autophagy is blocked, both pharmacological and genetic using the retina of mice deficient in autophagy Ambra1. These animals have alterations in the developing retina and accumulation of apoptotic cells. This accumulation is not due to a blockade in the expression of phagocytosis signals as previously observed in the chick retina after pharmacological inhibition of autophagy.

On the other hand, after the pharmacological inhibition of autophagy with 3-MA or wortmannin in mouse retinas *in vitro*, there is an accumulation of apoptotic cells due to decreased exposure of phosphatidylserine. Methylpiruvate supplementation, a permeable substrate for ATP production, restores the presentation of phosphatidylserine on the cell surface. This results in the reestablishment of the degradation of apoptotic cells. These data lead to think that autophagy has a cytoprotective role during embryonic development and that is necessary for the removal of apoptotic cells after cell death.

P01-8
Induction of human leukemic cells apoptosis by photoexcited fullerenes C_{60} is followed by remodelling of intracellular Ca^{2+} fluxes

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Changes in the level of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in integration with other signal-transduction cascades control cell proliferation, apoptosis, cell transformation and tumorigenesis. The question still remains how can cancer cells increase Ca^{2+} cycling to drive cell proliferation and to avoid Ca^{2+}-dependent apoptosis. It is assumed that altered expression of specific Ca^{2+} pumps and channels lead to reduced Ca^{2+} filling of endoplasmic reticulum (ER) and to Ca^{2+} release insufficient to produce apoptosis through excessive mitochondrial accumulation. Taking into account that many of Ca^{2+} transporters are sensitive to reactive oxygen species (ROS), redox regulation seems to be one of the ways to remodulate calcium homeostasis and to mediate cancer cell death. Recent progress in nanobiotechnology increased interest in biomedical application of fullerenes C_{60} – spherical carbon nanostructures, able to penetrate into the cells, to accommodate inside hydrophobic regions of cell membranes and to generate ROS with almost 100% quantum yield after photoactivation. We have demonstrated that pristine fullerenes C_{60} when combined with UV/Vis irradiation (λ = 320–600 nm) exhibit cytotoxic effect against human leukemic cells (L1210, Jurkat and MT-4). Cell death was registered at 24 hour and was proved by DNA fragmentation and caspase-3 activation to be of apoptotic type. No fullerene C_{60} phototoxicity was detected in normal T-cells (isolated Wistar rats thymocytes) while antileukemic drug cytosine arabinoside was cytotoxic for cells of both types. It is shown that ER store-operated Ca^{2+} entry (SOCE) in transformed cells is significantly lower than in normal. Using fluorescent dyes DCF-DA, indo-1 and TMRM we have demonstrated that leukemic cells respond to combined action of C_{60} and irradiation by continuous intensification of ROS production, $[Ca^{2+}]_i$ elevation, SOCE increasing and depletion of mitochondrial Ca^{2+} pool which precede cytocrome c release from mitochondria.

P01-9
The anticancer activity of Epothilone B in OV-90 cells – induction of apoptosis

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Objectives: We investigated the mode of cell death induced by Epothilone B in OV-90 ovarian cancer cell line derived from the malignant ascites of patients diagnosed with advanced disease. Epothilones form the new class of microtubule depolymerization inhibitors. These compounds were discovered in the early 1990s and data suggest that they have the potential to be more effective for the treatment of solid tumours than taxanes (paclitaxel (PTX) or docetaxel (DTX) which have been used in the clinic since 1992.
Methods: Cytotoxic activity of the drug was determined by the MTT assay. We measured also the level of apoptotic and necrotic cells, the production of reactive oxygen species (ROS) and changes in the mitochondrial membrane potential in human ovarian cancer cells exposed to Epo B in the presence and absence of an antioxidant, *N*-acetylcysteine (NAC).
Results: The studies confirmed that epothilone B was considerably more cytotoxic towards OV-90 cells than paclitaxel. Its IC_{50} concentration (70.46 ± 0.01 nM) was lower about three times than that of paclitaxel (199.53 ± 0.02 nM). It was found that the new drug induced both apoptosis and necrosis in the cells and this process was mediated by reactive oxygen species. Apoptotic cell death investigated by double staining with Hoechst 33258 and propidium iodide was associated with phosphatidylserine (PS) externalization, production of ROS and depolarization of mitochondrial membrane. The highest ROS level and also the lowest level of mitochondrial membrane potential (71.3% of control) were observed after 24 hour of incubation with Epo B. These effects were inhibited by antioxidant N-acetylcysteine.
Conclusions: Epo B is more potent than PTX in OV-90 cell line and it is able to induce of apoptosis mediated by reactive oxygen species.
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P01-10
TRAIL gene polymorphism and the risk of non-small cell lung cancer in turkish patients

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Lung cancer is a major public health problem throughout the world. Among the two major subtypes of small cell lung cancer and nonsmall cell lung cancer (NSCLC), 85% of tumors belong to the NSCLC histological types. Apoptosis is a fundamental biochemical cell-death pathway essential for normal tissue homeostasis, cellular differentiation, and development. TNF-related apoptosis inducing ligand (TRAIL) is important critical protein in extrensic and intrensic patways of apoptosis. Activation of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptor pathway is a promising therapeutic strategy to selectively eradicate cancer cells, including non-small cell lung cancer (NSCLC) cells. The aim of this study was to investigate the susceptibility and prognostic implications of the TRAIL in the non-small cell lung cancer. We investigated a single nucleotide polymorphism (SNP) in the 3-untranslated region of the TRAIL gene at position 1595 exon 5. We used PCR, RFLP and gel electrophoresis techniques to detect these polymorphism in 87 non-small cell lung cancer patiens and 87 healthy controls. We found no association between TRAIL genotypes and susceptibility of non-small cell lung cancer. The frequencies of the CC, CT and TT genotypes were 60.9%, 25.3%, 13.8% in NSCLC cancer patients and 55.2%, 36.8%, 8% in healthy controls, respectively. There were no association between tumor stages, lymph node metastasis and TRAIL genotypes but we found an increased risk in subjects carrying CT genotypes for the risk of advanced stage NSCLC but this difference was not statistically significant. These results suggest that the TRAIL polymorphism may not be associated with susceptibility to NSCLC.

P01-11
Cytotoxic effect of diclofenac in the melanoma cell lines A2058 and SAN

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Diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) widely used in clinical therapeutics, has cytotoxic effects and induces apoptosis in many cultured cell lines. Many studies suggest that NSAIDs, and in particular the highly selective cyclooxigenase-2 inhibitors, could act as anticancer. We have investigated the involvement of mitochondrial dysfunction in the mechanism of diclofenac-induced apoptosis in the melanoma cell lines, A2058 and SAN, and in the human fibroblasts immortalized cell line BJ5ta. The analysis by phase contrast microscopy of diclofenac-treated cells showed the typical morphologic changes related to apoptosis only in A2058 and SAN cell lines. These effects appeared after a treatment with 150 μM diclofenac suggesting a preferential cytotoxic effect in the two melanoma cell lines. Propidium iodide incorporation followed by cytometric analysis showed an increase of apoptosis during diclofenac treatment. Since the caspase 3 represents the final effector of the caspase dependent apoptotic process we have also analysed its activity following diclofenac treatment. A marked increase of caspase 3 activity in the cell extracts of A2058 and SAN cells was evidenced by spectrofluorimetric analysis as well as the analysis of intracellular levels of bcl-2 confirmed the pro-apoptotic effect of diclofenac only in the melanoma cell lines. Furthermore, the cytotoxic effect of diclofenac in the melanoma cell lines was associated to a decrease of the antioxidant SOD2 protein levels. These preliminary data suggest that mitochondria could represent a diclofenac target and in particular the reduction of SOD2 protein contributes to the mitochondria dysfunction.

P01-12
Effects of methylsulfonylmethane on viability of HCT-116 cell line

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Overview: Apoptosis is a genetically programmed cell death mechanism which plays important roles in normal physiology such as tissue homeostasis regulation and pathophysiology of various diseases. Defects in apoptotic gene and related protein expressions are closely related to progression of different types of cancer. Colorectal cancer, occurs due to uncontrolled growth of cells in the colon. Therefore, development and discovery of new chemical agents which may promote and/or manipulate the life span of these cancer cells have been a popular research area recent years.
Methods: Briefly HCT-116+/+ colon cancer cells were seeded to plates and treated with different doses of methylsulfonylmethane, after 24 hour MTT was added and absorbance at 690 nm was measured with spectrophotometry and the color intensity was used as an implication of cell viability. After treatments, cells were lysed with lysis buffer cocktail and spesific caspase-3 activity was spectrofluorometrically determined in cytosolic protein extracts using a specific substrate (Ac-DEVD-AMC) according to the supplier's instructions (Alexis Biochemicals).
Results and discussion: MSM decreased cell viability and increased caspase-3 activity in HCT116+/+ colon cancer cells at its 600 mM concentration. MSM can be used as an apoptotic agent in cancer, however further studies are needed to determine the mechanism of its effects and its optimum dose.

P01r-13
Inhibition of caspase-8 enhances anti-tumoral effects of vorinostat in endometrial cancer cell lines

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Background: Endometrial carcinoma (EC) is the fourth most common cancer in women from developed countries. Endometrial carcinomas had been classified into two pathogenetic groups: Endometroid and Nonendometroid type. The regulation of gene expression in many biological processes involves various epigenetic mechanisms. These epigenetic mechanisms include DNA and chromatin modifications by methylation, acetylation and phosphorylation. Epigenetic regulators such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) play an important role in gene expression and can modulate a variety of cellular functions that are involved in cell growth and survival.

Because aberrant histone acetylation is frequently observed in human cancers, HDACs are considered a promising target for cancer therapy and HDAC inhibitors are being developed.

Objective: This study was conducted to evaluate the efficacy of the histone deacetylase inhibitor vorinostat in the treatment of EC cell lines.

Results: Vorinostat treatment ofEC cells increased DNA damage and induce decrease in cell viability, proliferation and clonogenicity capacity in a dose- and time-dependent manner. Vorinostat significantly reduced BrdU incorporation in EC cells, which was associated with decreased cyclin D1 protein levels. Moreover, at high doses of Vorinostat caused an accumulation of cells in Sub-G1 phase and cleavage of the extrinsic and intrinsic initiator caspases -8 and -9 and the executioner caspases, suggesting induction of apoptotic cell death. Surprisingly, downregulation of caspase-8 expression by specific shRNAs or inhibitior of its proteolytic activity by the inhibitor IETD, enhanced Vorinostat-induced apoptosis. Apoptosis in response to Vorinostat or vorinostat plus inhibiton of caspase-8 was completely rescued by overexpression of BclxL protein.

Conclusions: Our study demonstrate that vorinostat triggers apoptosis in EC with participation. Both intrinsic and extrinsic caspases are activated by Vorinostat. Inhibitor on the extrinsic apoptotic initiator caspases increases the effectiveness of vorinostat to induce EC apoptosis.

P01-14
Inducing intracellular protein degradation via small molecules

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The intervention in biological processes by the use of small molecules is a widely applied concept that is not solely restricted to the inhibition of proteins. Enzyme inhibition, stabilization of variant proteins as well as the manipulation of enzymatic pathways are all common and attractive strategies for an artificial influence on intracellular processes and promising approaches for pharmaceutical and therapeutical research. However, the concept of small molecules lacks an important feature: not all types of proteins can be addressed using small molecules, thus only a fraction of the entire proteome is amenable. The remaining fraction of 'undruggable' types of proteins could not be targeted yet. Our latest research is focused on the possibility of developing small molecules that will bind and tag proteins independently of their class in order to induce apoptosis in cancer cells. Cancer cells are characterized by high levels of oxidative stress, which promotes the oxidation of redox-active cysteine residues to sulfenic acids. Thus, the large and structurally diverse sulfenome of cancer cells represents not only a group of proteins which is an excellent target for induced degradation, but also offers a possible application for the 'bind and tag' method. Herein the sulfenome is specifically targeted by an advanced small molecule ligand which chemoselectively forms covalent adducts and broadens the current hydrophobic tagging methodology and significantly affect the sulfenomes' mode of action as well as intracellular protein level.

P01-15
Autophagy as an active contributor to micronuclei clearance

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Autophagy (macroautophagy, *strictu sensu*) is known to participate in the quality control and turnover of cytoplasmic organelles, yet there is little evidence that macroautophagy targets nuclei in mammalian cells.

Studying the cellular perturbations arising after removal of several distinct cell cycle blockers (nocodazole, cytochalasin D, hydroxyurea or SP 600125), we noticed that cells manifested an increase in the frequency of micronuclei (positive for histone H2B-RFP) as well as an increase in autophagic puncta (positive for GFP-LC3) over several days. We decided to investigate whether those phenomena may be linked, if autophagy may target micronuclei (arisen as a result of deficient bipolar chromosome segregation in cells exposed to cell cycle perturbations).

A small but significant percentage of micronuclei co-localized with GFP-LC3 in autophagy-competent cells and this co-localization was lost after knockdown of ATG5 or ATG7. Electron microscopy analyses confirmed autophagic sequestration of micronuclei. 'Autophagic micronuclei' ($GFP\text{-}LC3^+$) were also decorated with p62/SQSTM1, while non-autophagic ($GFP\text{-}LC3^-$) micronuclei where p62/SQSTM1 negative. In addition, $GFP\text{-}LC3^+$ micronuclei exhibited signs of envelope degradation and $\gamma H2AX^+$ DNA damage foci, yet stained less intensively for chromatin markers, whereas $GFP\text{-}LC3^-$ micronuclei were surrounded by an intact envelope and rarely exhibited markers of DNA damage.

These results indicate that micronuclei can be subjected to autophagic degradation. Moreover, it can be speculated that removal of micronuclei may contribute to the genome-stabilizing effects of autophagy.

P01-16
Rheum ribes ethanol extracts induce apoptosis of HL-60 cells by a caspase dependent pathway

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The effects of ethanol root extract (ERE) of *Rheum ribes* L. on the proliferation and apoptosis of Human Myeloid Leukemia (HL – 60) cells were investigated for the first time. Dried and pulverized plant samples were extracted by ethanol at a ratio of 1:12 (w/v) at 50°C for 24 hour. HL – 60 cells were cultured in the presence of various concentrations of ERE up to 72 hour. The cell viability was determined by XTT. ERE inhibited the survival of HL-60 cells in a concentration- and time-dependent manner. EC_{50} value of ERE was calculated as 128.15 ± 0.54 μg/ml. Furthermore, *R. ribes* caused HL-60 cell shrinkage and formation of apoptotic body, which are typical characteristics of apoptotic cell death. In addition, *R. ribes* caused HL-60 cells apoptosis in

an concentration and time dependent manner via formation of phosphatidylserine externalization, as evidenced by flow cytometry. Exposure of HL-60 cells to 500 μg/ml ERE for 72 hour resulted in a shift of 87% of the cell population from normal to the early/late apoptotic/necrotic stage. The *R. ribes* -induced apoptosis may be partially attributed to the activation of caspase-3, since its activity was increased 2.9 fold in 18 hour treatment, and up-regulation of caspase-3 expression was detected in western blot. The significant release of cytochrome c from the mitochondria into the cytosol was observed. The mRNA expression ratio of Bax/Bcl-2 was increased. The apoptosis caused by *R. ribes* was also demonstrated by DNA ladder and TUNEL. Furthermore, the *R. ribes* extracts were revealed to be a potential scavenger of DPPH radicals (EC_{50}: 16.31 ± 2.41 μg/ml) and the chemical composition of the extracts was quantified by colorimetric determination of total phenol (226.61 ± 10.65 mg Gallic Acid Equivalent/g dried extract) and flavonoid (70.89 ± 6.84 mg Catechin Equivalent/g dried extract) contents. These findings suggest that *Rheum ribes* exhibits potential antioxidant and anticancer properties by inducing caspase-dependent cell death.

P01-17
Oxidative stress and proapoptotic virtue of highly expressed Bcl-2 in Korbazol-induced apoptosis of leukemia cells

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Objectives: Korbazol is a natural product that has recently been described to exert strong cytotoxic effect on B-CLL cells *in vitro*, but not in the normal mononuclear cells. We have demonstrated that Korbazol causes endoplasmic reticulum (ER) stress, activation of caspases-2 and -4, mobilisation of intracellular Ca^{2+} and subsequent apoptotic cell death without engagement of mitochondria and cytochrome c release into the cytosol.
Methods: The aim of this study was to investigate engagement of oxidative stress in Korbazol-induced apoptosis and expression levels of Bcl-2, as modulator of cell death being involved in regulation of ER and mitochondrial membranes integrity. The concentration of O_2^-, H_2O_2 and activity of superoxide dismutase and glutathione peroxidase in the samples were measured by spectrophotometric method. Expression of Bcl-2 was determined by Western blot analysis.
Results: We have demonstrated that Korbazol increased concentration of $O2^-$ and H_2O_2 through inhibition of SOD and Gpx activity, thus inducing oxidative stress. Addition of antioxidative enzymes decreased Korbazol cytotoxicity for almost 50%. Western blot analysis of Bcl-2 have shown highly elevated levels of this protein after treatment with Korbazol. Pretreatment with HA 14-1, Bcl-2 ligand which antagonizes the function of Bcl-2, reduced cell death for 25%.
Conclusions: These results point to involvement of reactive oxygen species concomitant to ER stress in Korbazol-induced apoptosis. The role of Bcl-2 in these events remains to be elucidated. Although it is well known that overexpression of Bcl-2 protects cells from apoptosis, there are a number of reports indicating that high cellular concentrations of Bcl-2 lead to induction of apoptosis via the caspase cascade.
Keywords: Korbazol; Apoptosis; Oxidative stress; Bcl-2

P01-18
The role of WT1+/+ and WT1+/− isoforms in breast cancer

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Wilms' Tumor 1 (*WT1*) gene is expressed in wide variety of solid cancers. High levels of the WT1 either mRNA or protein correlated with aggressive phenotypes in breast cancer. Our present study demonstrated that the *17AA(−)* mRNA alone expressed in adjacent normal breast tissue at 43.33% while it does not expressed alone in infiltrating breast carcinoma. The mix expression of *17AA(+)* and *17AA(−)* mRNA highly expressed in breast carcinoma grade 1, 2 and breast carcinoma grade 3 tissues at 75% and 81.82%, respectively. Similarly, mix 17AA(+) and 17AA(–) protein highly expressed in breast carcinoma grade 1, 2 and 3 tissues while 17AA(–) highly expression in adjacent normal breast tissue. Since, alternative splicing of WT1 transcript generates four major protein isoforms, each having different functional properties, WT1+/–, WT1+/–, WT1–/+ and WT1–/– were constructed and transfected into MCF-7 cell. These four isoforms transcript encodes the exon1 to exon10 but different in exon 5 (17AA + or –) and exon 9 (KTS + or –). Overexpression of WT1+/+ and WT1+/– related to the high expression of ER and Her2. Then, we further performed the siRNA experiment to knock down WT1. Interestingly, silencing of both WT1 isoforms WT1+/+ and WT1+/– resulted in the decreasing of both ER and HER2. These results indicate that WT1+/+ and WT1+/– play a crucial oncogenic role in breast cancer and might be involved in cancer development and progression. In addition, overexpression of WT1+/+ and WT1+/- protected cells from cell death induced by apoptosis-inducing agent, doxorubicin. The expression of proapoptotic Bak and caspase-7 was decreased by the expression of both WT1 isoforms. Taken together, these results indicated that WT1+/+ and WT1+/– isoforms function as anti-apoptotic protein.

P01r-19
Cytochrome *c*: an old protein controlling life and death since the dawn of time

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Cytochrome *c* (C*c*), a small soluble hemeprotein, is highly conserved along evolution. In mammals, C*c* plays a dual role in cell life and death. Under homeostatic conditions, C*c* is retained inside the mitochondria and acts as an electron shuttle in the electron transfer respiratory chain. Upon apoptotic stimuli, however, C*c* is released into the cytoplasm so as to serve as an essential key factor by binding to Apaf-1 and further assembling the apoptosome, the machinery responsible for the activation of caspases (1). The mitochondria-to-cytoplasm C*c* translocation has been long considered as a random event, although it is an evolutionarily conserved process even in organisms in which the apoptosome assembly is independent of C*c* or in which the apoptosome is missing. These findings, along with the fact that apoptosis remains active in Apaf-1 knockout mutants but not in C*c* knockout mutants (2,3), lead one to wonder if cytoplasmic C*c* could play other putative signaling functions.

To better understand the role of C*c* in the onset of apoptosis and to harmonize the different phenotypes of Apaf-1 and C*c* knockout mutants, we have developed a proteomic approach

based on affinity chromatography with human or plant C*c* as bait. A total of 24 and 11 C*c* partners in human and plant cell extracts, respectively, have been identified. Their *in vivo* interaction with C*c* and cellular localization were further analyzed by BiFC. Altogether, our results open a new way to understand the C*c*-dependent activation and progression of programmed cell death in plant and human cells.

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P01-20
Investigating the effects of altered Bag-1 expression on Mcf-7 human breast cancer cell lines

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Bag-1 (Bcl-2 associated athono gene-1) belongs to an anti-apoptotic Bag family that acts as an adaptor protein to regulate a wide variety of cellular processes, including proliferation, cell survival, transcription, apoptosis and motility. However, the molecular pathways by which Bag-1 carries out these functions are still unclear. Bag-1 has four functionally distinct isoforms that can interact with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. To understand Bag-1 and its isoforms involvement in anti-apoptotic processes in Mcf-7 breast cancer cells, we silenced Bag-1 gene using Bag-1 siRNA. We first analyzed Bag-1 silencing effect on different apoptotic genes by qRT-PCR, and later studied these related proteins expression levels using immunoblotting. Bag-1 silencing caused a decrease in Akt gene expression while it elevated Bcl-2, C-Raf and 14-3-3theta genes expression levels. Immunoblotting results showed that Akt activation, which is a marker for cell survival, was lost by Bag-1 siRNA transfection, revealing complementarity between mRNA and protein levels. However, studies on protein level analysis for C-Raf and Bcl-2 showed a significant downregulation of these proteins after Bag-1 silencing. Similar variations between Bag-1 interacting proteins' mRNA and protein expression levels are observed after Bag-1 silencing for different cell lines, suggesting possible post-translational modifications during Bag-1 mRNA processing. We also studied the alterations on other pro-and anti-apoptotic proteins expression level with Bag-1 silencing to further understand the molecular details of Bag-1 involved apoptotic mechanism. We believe that once the molecules in the Bag-1 related apoptotic pathway is delineated, Bag-1 might be used as a marker for cancer studies both for treatment and prognosis.

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P01-21
Apoptosis of ganglioside-deficient cells (GM95 and DonQ) exposed to different concentrations of *Clostridium* α-toxin

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Clostridium perfringens phospholipase C (Cp-PLC), also called α-toxin, is considered a prototype of cytotoxic bacterial phospholipases C, and several facts indicate that it is the major virulence factor in *C. perfringens*-induced gas gangrene (1), an acute and devastating soft tissue infection characterized by gas accumulation at the infection site, severe edema and myonecrosis (2). If left untreated, gas gangrene is always fatal due to the toxaemia, disruption of cardiac function and shock (2). Different results show that Cp-PLC hydrolyses phosphatidylcholine and sphingomyelin at a broad range of pH, induces platelet aggregation, and is hemolytic, cytotoxic, myotoxic and lethal (3). Considerable progress has been made during recent years in the knowledge of the mechanism by which the toxin induces platelet aggregation and hemolysis. The understanding of the mechanism of toxicity in nucleated cells is incomplete (3). It is known that at high concentrations the toxin causes membrane disruption and cytolysis. However at low concentrations α-toxin causes only limited hydrolysis of its substrates leading to the unregulated generation of bioactive lipids and inducing cell death independently of plasma membrane disruption (4). Moreover, ganglioside-deficient cells, compared with wild type, show a high sensitivity to the toxin and undergo apoptosis upon exposure to very low concentrations of toxin (5). The accumulated data support the hypothesis that α-toxin undergoes endocytosis via a caveolar-like mechanism and cleaves the lipids of the membranes from the endolysosomal system which somehow triggers apoptosis. Indeed, our unpublished results show that inhibitors of caveolae-dependent endocytosis such as methyl-β-cyclodextrin, filipin and nystatin protect against the apoptotic effect of toxin. The aim of this work will be to quantify the apoptosis in ganglioside-deficient cells (GM95 and DonQ) exposed to different concentrations of α-toxin, characterize the changes in lipid composition and correlate those changes with the induction of apoptosis. The knowledge obtained will provide new insights towards understanding the mechanism of toxicity of bacterial phospholipases C similar to α-toxin and could help to develop novel therapeutic strategies in gas gangrene and other bacterial infections.

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P01r-22
E2F1 and Chk1 play an important role in the resistance of melanoma to methotrexate

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Melanoma is not only the most aggressive form of skin cancer, but also it's notoriously resistant to all current modalities of cancer therapy, including the drug methotrexate (MTX). Recently our laboratory has described a melanoma-specific mechanism of resistance to MTX by which folate receptor a (FRa)-mediated endocytotic transport of MTX leads to the sequestration of this drug within melanosomes and its exportation outside the cell. Thus, this process reduces the accumulation of MTX in intracellular compartments and makes it behaves as a cytostatic agent rather than a cytotoxic drug, but the way by which MTX induces cell cycle arrest remains unknown. Because the ability of cells to delay cell cycle progression and halt DNA synthesis represents a defensive mechanism that spares potential toxicity, in this study we focused on deciphering the mechanisms underlying that cell cycle arrest. Thereby, we identify E2F1 and checkpoint kinase 1 (Chk1) as key mediators of this mechanism of resistance. The results indicated that MTX stimulated the transcriptional activity

of E2F1 on the promoters of dihydrofolate reductase and thymidylate synthase, which lead to an increase in the dTTP levels instead of dTTP depletion, as occurs in MTX-sensible cells. Since dTTP is an allosteric inhibitor of ribonucleotide reductase, dTTP excess induced DNA replication fork stress that activates the ATR/Chk1 checkpoint. Under these conditions, melanoma cells were protected form apoptosis by arresting their cell cycle in S phase. However, abrogation of this checkpoint by Chk1 silencing, rapidly triggered cell death after MTX treatment, suggesting that inhibition of Chk1 in combination with this kind of antimetabolite chemotherapy is a viable therapeutic strategy to overcome melanoma resistance.

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P01r-23
Resistance to butyrate impairs bile acid-induced apoptosis in human colon adenocarcinoma cells via up-regulation of Bcl-2

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Environmental factors are strongly involved in the development of colon cancer. Among them, the continuous exposure to high concentrations of bile acids in individuals with a fat-rich diet leads to DNA damage and may allow selective growth of cells resistant to the cytotoxic effects of these agents, increasing the risk of tumor development. We have previously reported that DCA and CDCA trigger apoptosis in BCS-TC2 human colon adenocarcinoma cells through the activation of membrane-associated enzymes [NAD(P)H oxidases and PLA_2], which leads to oxidative stress that eventually triggers the mitochondrial apoptotic pathway. The activation of caspase-3 by the apoptosome activates Bax via cleavage of Bcl-2, thus generating a feedback loop that amplifies the apoptotic signal.

On the other hand, BCS-TC2.BR2 butyrate-resistant cells, that show resistance towards different types of stress, are still sensitive to bile acid-induced apoptosis through a similar mechanism to that observed in their parental cells. However, BCS-TC2.BR2 cells express higher levels of the antiapoptotic protein Bcl-2 that prevent Bax activation overcoming the pro-apoptotic feedback loop observed in BCS-TC2 cells. After activation of the intrinsic apoptotic program by oxidative stress, caspase-3 is not able to degrade enough Bcl-2 protein to allow the release of Bax, the formation of Bax-dependent pores in the mitochondrial membrane and the subsequent release of proapoptotic factors. Moreover, this resistance can be reverted *in vitro* by the use of the Bcl-2 inhibitors, suggesting the potential clinical use of these inhibitors in patients with tumors with high Bcl-2 levels.

P01-24
The association of oncogenic role between WT1 and p53 in breast cancer

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The Wilms' tumor 1 or WT1 was initially demonstrated from childhood kidney cancer. WT1 plays an important role in the pathogenesis and progression of leukemia. However, the mechanism of WT1 in breast cancer remains doubtful. Furthermore, the cellular condition of p53 affects the capability of WT1 to function as tumor suppressor or oncogene. Hence, in this study, the relationship between WT1 and p53 was elucidated in breast cancer cell lines: MCF-7 (p53 wild type) and MDA-MB-231 (p53 mutation). siRNA transfection has been used to study the function of WT1 and p53. Trypan blue dye assay was performed to examine a percentage of death cells in total cells. Involved proteins were detected by Western blot analysis. MCF-7 and MDA-MB-231 overexpressing cell of all 4 isoforms of WT1 (WT1+/+, ±, −/+, −/−) have been constructed. Transfection of $siRNA_{WT1}$ into MCF-7 and MDA-MB-231 cells resulted in reduction of WT1 level in both cells. These evidences induced an increasing in number of cell deaths compared with $siRNA_{neg}$ transfected cell as a negative control. A reducing in WT1 level also increased the expression level of p53, p21 in MCF-7 but provided some controversy result in MDA-MB-231. MCF-7 and MDA-MB-231 overexpressing cell of all 4 isoforms of WT1 tended to have the different feature of each WT1 isoform. The growth assay of all 8 overexpressing cells and their parental cells were recorded. MCF-7/WT1−/− represented the highest growth whereas MCF-7/WT1+/− showed the lowest one. In contrast, MDA-MB-231/WT1+/− showed the prominent profile compared to MDA-MB-231/WT1−/− tends to expand at the lowest rate. These results implied that WT1 isoforms possesses different character in breast cancer cell lines and theirs effect depends on the status of p53 in the cells. WT1 acted as an oncogenic role in breast cancer when cellular condition of p53 was wild type.

P01-25
Implication of p38MAPK in colorectal cancer therapy: breaking the balance between apoptois and autophagy

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5-fluorouracil, is one of the most important pharmacological agents in the treatment of colorectal cancer. However no role has been established in 5-fluorouracil response for MAPKs. Here we demonstrate that p38MAPK activation is a key determinant in the cellular response to 5-fluorouracil. Thus, inhibition of p38MAPK by chemical and genetic approaches correlates with a decrease in the 5-fluorouracil-associated apoptosis and chemical resistance. Activation of p38MAPK by 5-fluorouracil was dependent on ATM and ATR, showed a redundancy of function, trough MAP2K, MKK3 and MKK6. Resistance associated with p38MAPK inhibition correlates with an autophagic response that was mediated by a decrease in p53-driven apoptosis without effect onto p53 dependent autophagy as the evaluation of DRAM and Setrin2 supports. Moreover, the results with colorectal cancer derived cell lines with different p53 status and patterns of resistance to 5-FU as HT-29, RKO, SW620, LoVo. Furthermore, our observation could apply to other contexts as in the case of acquire resistance or in the radio sensitizer effects of 5-fu. In summary, our data demonstrate that p38MAPK signaling pathway is controlling the balance between apoptosis and autophagy in response to the genotoxic stress triggered by 5-fluorouracil.

P01-26
Mechanisms of leptin action on placental cell survival

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Fetal-maternal dialogue during implantation involves multiple regulators such as leptin. This 16KD protein plays diverse roles in placental growth and survival.

Previous results from our group demonstrated that leptin increases cell proliferation and survival in JEG-3 and BeWo cells. We also demonstrated that leptin expression is tightly regulated by different placental regulators.

Objectives: The aim of the present work is to study the mechanisms involved in leptin rol on placental apoptosis.

Methods: BeWo and Swan cells, and human term placental explants were used. Western blot analyses were carried out to detect leptin, caspase 3, Bcl-2, Bax and p53 expression. Transfection assays with reporter constructs were used to determine leptin effect on different transduction pathways

Results: Leptin treatment diminished the proteolysis of caspase-3 in a dose dependent manner. Moreover the diminution in endogenous leptin by treatment with an antisense oligonucleotide (2–4 μM) increases cellular apoptosis measured by caspase-3 activation. Bcl-2 and Bax levels were determined after leptin treatment and the relationship between them calculated. Leptin enhanced Bcl2/Bax relation. On the other hand leptin diminished the expression of a reporter plasmid containing Bax promoter. The expression of the key cell cycle regulator p53 was also determined. Leptin diminished p53 protein level dose dependently.

Conclusions: All these results reinforce the notion of leptin as a placental cytokine with the function of promoting survival of placental cells.

P01r-27
Calpains mediate nuclear pore complex degradation during mammary gland involution

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Post-lactational mammary gland involution is characterized by extensive death of the secretory epithelium coupled with remodeling of the extracellular matrix and adipogenesis to regenerate the fat pad. The expression and activity of calpain-1 and -2, calcium-dependent proteases, were increased during weaning. This fits with increased intracellular Ca^{+2} levels within the gland. We have demonstrated that calpains, upon activation, translocate to lysosomal and mitochondrial membranes, being involved on their permeabilization. Proteins from the nuclear membrane, such as several components of the nuclear pore complex (NPC) are possible targets to be cleaved during apoptosis. We hypothesized that calpains could also translocate to the nuclear membrane, promoting the degradation of NPC proteins. Calpain-mediated cleavage of the NPC would induce the lost of the selective transport across the nuclear membrane. Here we show that during involution a time-dependent translocation of calpains to the nuclear membrane occurs, with subsequent degradation of several nucleoporins: Nup 62, Nup 98, Nup 153, etc. This nuclear pore degradation allows the accumulation of large proteins within the nucleus such as tubulin or CAD (caspase- activated deoxyribonuclease), the latter being responsible of the DNA fragmentation observed during apoptosis. To confirm this hypothesis, calpain inhibitors (calpeptin or *Capn1* siRNA) were administered '*in vivo*'. After calpain inhibition, nucleoporin cleavage was prevented and mammary gland involution delayed. These results suggest a new role for the calpain system in nuclei, providing new insights into the physiological pathways that control the fate of mammary epithelial cells during involution.

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P01-28
TGF-beta and epithelial-mesenchymal transition (EMT): a step forward to apoptosis-resistance and stemness phenotype

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Transforming Growth Factor-beta (TGF-β) suppresses early stages of tumour development, but it later contributes to tumour progression when cells become resistant to its suppressive effects. In liver cells, TGF-β plays a dual role in the control of apoptosis. On one side, it mediates cell death by a mitochondrial-dependent mechanism (1). On another side, it induces anti-apoptotic signals through activation of the EGF receptor pathway (2). Cells that survive to the apoptotic effects of TGF-β undergo Epithelial-Mesenchymal Transition (EMT), which mediates acquisition of motility and scattering properties and, additionally, confers cell resistance to apoptosis (3). A key master gene involved in this response is Snai1, which impairs the TGF-β-induced, mitochondrial-mediated, cell death and is sufficient to induce EMT in adult hepatocytes, cells otherwise refractory to this transition upon exposure to TGF-β (4). Interestingly, after chronic treatment of liver cells with TGF-b, they not only show a mesenchymal and migratory, but also a de-differentiated phenotype, acquiring properties of stem cells (5). Furthermore, chronic *in vitro* TGF-β treatment changes the tumorigenic potential of hepatoma cells. Tumor growth is similar, but phenotype of lesions reflects an apoptosis resistant and stem-like phenotype, which provokes the appearance of less differentiated tumors (hepatoblastomas) or transdifferentiation to a different liver tumor lineage (cholangiocarcinoma). Together, our work points to a role of EMT in overcoming TGF-β tumour-suppressor effects in hepatocytes, and particularly in liver cancer cells, switching the response from tumour suppression to tumour progression, making them resistant to cell death and prone to acquire migratory/invasive and stem cell properties.

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P01-29
Biological properties of a lipophilic extract of sea-star *Marthasterias glacialis* L. upon several cancer cell lines

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The sea has been increasingly regarded as an excellent source of new drugs for human health, mainly due to the remarkable chemical diversity found in marine organisms.

In this work, the effect of a lipofilic extract of the sea-star *Marthasterias glacialis* L. was studied on the human cell lines Caco-2 (colon cancer), MCF-7 (breast cancer) and SH-SY5Y (neuroblastoma).

Metabolite profiling of the extract revealed the presence of several saturated and unsaturated fatty acids and sterols, with palmitic, arachidonic acid and eicosenoic acid derivatives being the most significant ones. Overall, the purified fraction of the lipidome caused several morphological changes upon the cell lines tested, including the advent of cytoplasmatic vesicles. Transmission electron microscopy analysis and specific cytoplasmatic stains identified these structures as adiposomes.

Apart from marked morphological changes, which also included chromatin condensation and cell shrinkage, the purified lipophilic extract from *M. glacialis* caused loss of cell viability, inhibition of DNA synthesis and cell cycle arrest. Remarkably, this effect was highly dependent on the cell line used, with the neuroblastoma cell line being the most susceptible one. During this process, no loss of membrane integrity was noticed which, together with increased values of caspase-3 activation, suggests a programmed process of cell death, putatively apoptosis. The effect of this organism's lipid constituents upon the homeostasis of the neuroblastoma cell line and its association with the induction of apoptosis will be discussed.

P01-30
p53-independent apoptotic responses in alveolar type II cells treated with air pollution-derived particulate matter

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Air pollution is a serious health problem, worsening respiratory, allergic and cardiovascular diseases, besides being associated to lung cancer. Despite the efforts of Brazilian environmental agencies in establishing regulations which decreased pollutant emissions by motor vehicles, the increasing number of vehicles in large cities is still responsible for high levels of air pollution. Among the air pollutants, particulate matter is of particular interest due to its major contribution to the health effects attributed to air pollution. The objective of this study was to evaluate the effects of particulate matter (PM) from different sources on apoptosis in immortalized human alveolar type II cells (A549). PM induced dose and time-dependent apoptosis, measured by quantification of sub-diploid nuclei using FACS. Apoptosis induced by PM derived from diesel exhausts was increased in cells pre-treated with the ATM/ATR kinases inhibitor caffeine. These kinases are known to phosphorylate and activate p53 in response to some types of stress. However, cells treated with diesel-derived PM did not phosphorylate or activate p53, as evaluated by Western Blot. Despite the absence of p53 activation, increased expression of pro-apoptotic Bcl-2 family members Noxa and Puma was observed on cells treated with diesel-derived PM, as assessed by Real-Time PCR. We also found increased expression of Fas, determined by Real-Time PCR and western-blot. We are currently studying the specific role of these proteins on PM-induced apoptosis using RNAi. Results from these studies may help to understand the process of cell death in the lung of individuals exposed to air pollution.

P01-31
Apoptotic phosphorylation of histone H3 on Ser-10 by protein kinase Cδ

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Phosphorylation of histone H3 on Ser-10 is regarded as an epigenetic mitotic marker and is tightly correlated with chromosome condensation during both mitosis and meiosis. However, it was also reported that histone H3 Ser-10 phosphorylation occurs when cells are exposed to various death stimuli, suggesting a potential role in the regulation of apoptosis. Here we report that histone H3 Ser-10 phosphorylation is mediated by the pro-apoptotic kinase protein kinase C (PKC) δ during apoptosis. We observed that PKCδ robustly phosphorylates histone H3 on Ser-10 both *in vitro* and *in vivo*. Ectopic expression of catalytically active PKCδ efficiently induces condensed chromatin structure in the nucleus. We also discovered that activation of PKCδ is required for histone H3 Ser-10 phosphorylation after treatment with DNA damaging agents during apoptosis. Collectively, these findings suggest that PKCδ is the kinase responsible for histone H3 Ser-10 phosphoryation during apoptosis and thus contributes to chromatin condensation together with other apoptosis-related histone modifications. As a result, histone H3 Ser-10 phosphorylation can be designated a new 'apoptotic histone code' mediated by PKCδ.

P01r-32
Calpains are involved in lysosomal and mitochondrial leakage in epithelial cell death during mammary gland involution after lactation

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Calpains are implicated in physiological processes, such as cytoskeletal remodeling, cellular signaling and apoptosis. We examined the role of ubiquitous calpains (calpain-1 and -2) in mammary gland involution. Post-lactational regression of the mammary gland is characterized by a loss of secretory epithelial cells, collapse of alveolar structures and tissue remodeling. Calpain expression was increased during weaning; this evidence, together with increased Ca^{2+} within the gland, leads to the activation of calpains. After weaning, isolated mitochondria and lysosomes showed an increased activity of both calpains. While

calpain activation could trigger the release of cytochrome c and other pro-apoptotic factors in mitochondria, this activation might be essential, in lysosomes, for tissue remodeling by releasing cathepsins into the cytosol. During weaning, calpains translocate to the lysosomes processing membrane proteins. To identify these substrates, lysosomal fractions were treated with recombinant calpain and cleaved products were identified by 2D-DIGE. Both the lysosomal associated membrane protein 2a (LAMP2a) and the subunit b_2 of the v-type H^+ ATPase were proteolyzed by calpains. *In vitro* experiments showed that Lamp2a cleavage was calpain dose-dependent and several inhibitors prevented it. *In vivo* administration of the calpain inhibitor, calpeptin, to 72 hour weaned mice attenuated calpain activation, decreased LAMP2a cleavage and DNA fragmentation, and delayed tissue remodeling during involution. These results suggest that calpains are responsible for mitochondrial and lysosomal membrane permeabilization, supporting the idea that lysosomal-mediated cell death is a new hallmark of mammary gland involution. BFU2010-18253; GVPROMETEO 2010-075; PS09-02360 & AP 085/11.

P01-33
Evaluating the membrane topology of BAK and the role of selected mitochondrial lipids in BAK-driven MOMP

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Mitochondrial outer membrane permeabilisation (MOM) permeabilization is the point of no return in many forms of apoptotic cell death. The lethal effect of MOM permeabilization is twofold, as it allows activation of pro-apoptotic caspase enzymes and also damages normal mitochondrial function. The BCL-2 family members BAX/BAK are crucial MOM protein which shifts from an 'inactive' to an 'active' conformation upon apoptosis triggering. It is well established that functional BAK activation involves profound conformational changes in the protein, and that BAK itself is part of the apoptotic pore. However, important aspects of the BAK activation and action processes remain unknown. Chief among these is elucidating the topology of MOM-anchored BAK at different stages of the apoptotic process. To achieve this goal, we are developing different approach for dissecting the location of individual BAK residues at the MOM level, based on the substituted cysteine accessibility method (SCAM^{TM}). We are also analyzing the potential role of specific mitochondrial lipids in BAX/BAK activation and action.

P01-34
KAISO, a unique modifier of the p53 code, is a master regulator of apoptosis

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KAISO, previously described as a binding protein of methylated DNA and repressor of transcription, of which function and action mechanism are poorly understood. We showed here that DNA damage induces KAISO expression prior to p53 expression, and that KAISO interacts with p53 and p300 to from a complex. The interaction alters the acetylation code of p53 by p300. KAISO increases acetylation of the two key lysine residues K320 and K382, but specifically inhibits acetylation of K381. The p53 code considerably increase not only p53 stability by inhibiting ubiquitination and degradation, but also p53 target DNA binding activity and specificity to apoptotic gene promoters. With the newly coded p53 and p300, KAISO increases expression of *p21WAF/CDKN1A* and thegenes of apoptotic pathways. In *Kaiso* knockout MEFs and mice, both the intrinsic and extrinsic apoptosis pathways are disrupted and cells proliferate rapidly. KAISO, as a master regulator of apoptosis, directs the primary cellular defense system against genotoxic challenges in mammals.

P01-35
Silencing of the transcription factor STAT3 sensitizes lung cancer cells to DNA damaging drugs, but not to TNFSÁ- and NK cytotoxicity

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STAT3 (Signal Transducers and Activators of Transcription 3) is atranscription factor constitutively activated in a large number of human tumors. Persistent signaling via STAT3 may contribute to tumor progresión by stimulating cell proliferation, preventing apoptosis, modulating immune responses and angiogenesis. Therefore, inhibition of the pro-survival STAT3 activity may be a good strategy for sensitization cancer cells to DNA-damaging chemotherapeutics or immune cytotoxicity. We silenced STAT3 expression in human non-small lung carcinoma A549 cells to elucidate its role in lung cancer cell survival, resistance to chemotherapeutics, TNFSÁ and natural killer (NK) cell-mediated cytotoxicity. Silencing of STAT3 using plasmid-transcribed specific shRNAs did not impair survival and proliferation of A549 cancer cells, suggesting that STAT3 is not essential for basal survival and cell growth. On the other hand, stable silencing of STAT3 sensitized lung cancer A549 cells to doxorubicin and cisplatin treatments in p53 independent manner. The sensitization could be mediated through its ability to regulate Bcl-xL expression. In contrast, STAT3 depletion did not modulate TNFSÁ cytotoxicity and NK-mediated cell death. We demonstrated that STAT3 depletion increased NFSEB activity, and treatment with TNFSÁ, but not doxorubicin enhanced this effect, likely providing a compensatory, pro-survival signal. Altogether, our data suggests that STAT3 is not implicated in the control of basal proliferation and survival of lung carcinoma cells, but can modulate susceptibility to DNA damaging chemotherapeutics by inducing cell death via regulation of intrinsic apoptotic pathways.

P01-36
Seminal plasma proteins prevent apoptosis in ram spermatozoa

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We have previously shown that seminal plasma proteins (SPP) are able to protect and repair the cold-shock damage to ram

spermatozoa. In this study we analyze the protective effect of SPP against refrigeration and induced-apoptosis. Seminal plasma free ram spermatozoa were incubated for 15 min with 1.7 mg/ml of SPP at RT. Refrigeration (15°C, 30 min) and induction of apoptosis (betulinic acid [BA] or Fas ligand [FasL]) were performed, and viability (PI/CFDA staining), motility and mitochondrial membrane potential (Ψm, MitoTracker Deep Red/Yo-Pro), phosphatidylserine translocation ([PS], annexinV/CFDA), DNA damage (TUNEL assay) and caspase-3 and -7 activity (Vybrant FAM Assay Kit) were evaluated by flow cytometry. The proportion of membrane-intact spermatozoa in samples incubated with BA (46.3 ± 6%) or refrigerated (57.2 ± 6.2%) increased up to 56 ± 5.6% and 64.5 ± 8.9%, respectively, in the presence of SPP. The addition of SPP also preserved higher values ($p < 0.001$) of both total and progressive motility and prevented the loss of Ψm induced by BA and FasL ($p < 0.01$). PS translocation BA- (52 ± 9.7%), FasL- (55 ± 6.7%) or refrigerated (41.5 ± 3.8%) samples, decreased ($p < 0.05$) in the presence of PPS to 19 ± 3%, 36.8 ± 5% or 14.5 ± 4.6%, respectively. The addition of SPP before treatments resulted in decreased caspase activity ($p < 0.05$) and lower ($p < 0.01$) numbers of DNA-damaged sperm. The results obtained indicate that SPP are able to protect ram spermatozoa against refrigeration and apoptosis.

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P01-37
2-Arachidonoyglycerol (2-AG) and placental development: role of endocannabinoids in cytotrophoblast cells turnover

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The connection between cannabis consumption and negative consequences in reproduction is established for years, though only recently it has been demonstrated the importance of endocannabinoid signalling in reproductive events and the relation of its anomalies and infertility and miscarriages. In spite of being identified in human placenta, the role of the endocannabinoid 2-arachidonoylglycerol (2-AG) in placental development is still an incognita. We have previously demonstrated that low concentrations of 2-AG induced apoptosis in decidual cells, which suggests a role in decidua remodelling. Here we intend to evaluate the effects of 2-AG in BeWo cells, a widely used model of cytotrophoblasts of first trimester placenta. We determined the effects of 2-AG in trophoblast cells viability and proliferation by MTT assay, LDH release and ^{3}[H]-thymidine incorporation assay, in a range of concentrations between 0.01 and 50 μM for 24, 48 and 72 hour treatments. Morphological alterations were assessed by phase contrast microscopy, Giemsa and Hoechst staining, whereas the generation of oxidative and nitrative stress was evaluated by fluorimetry. It was verified that 2-AG induces a decrease in cell viability and DNA synthesis in a time and concentration-dependent manner. The pre-treatment with AM630, a CB2 antagonist was able to revert those effects. No significant LDH release was detected at concentrations of 15 μM or below. The treated cells presented morphologic alterations such as chromatin condensation, cell shrinkage and apoptotic bodies. Additionally, 2-AG induced a significant increase of reactive species of oxygen and nitrogen. Together, these results suggest 2-AG as a novel intervener into the trophoblast cells turnover.

P01-38
Ectopic expression of an activated Her2/ERBB2 oncogene in human breast epithelial cells confers sensitivity to endoplasmic reticulum (ER) stress-induced apoptosis

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Cancer cells are subject to stressful conditions in their tumor microenvironment, including low oxygen supply, nutrient deprivation and pH changes. All these stresses activate a range of cellular stress response pathways, including the unfolded protein response (UPR) which seems to play an important role in tumorigenesis. Our results show that human breast epithelial cells that over-express the Her2/ERBB2 oncogene are very sensitive to agents inducing ER stress. In order to characterize the underlying mechanism of this enhanced sensitivity to ER stress, we have generated a breast epithelial cell line MCF10A expressing a constitutive active form of the oncogen Her2/ERBB2. We have observed that expression of the Her2/ERBB2 oncogen causes a deregulation of the MAPK/ERK and PI3K/AKT pathways which are involved in the differential response of the UPR to ER stress agents and the resulting cell death. Finally, we demonstrate that PERK/ATF4 pathway is implicated in this cell death because the inhibition of this UPR branch by RNA interference blocks the apoptosis program activated by ER stress-inducing agents in these cells.

P01-39
Transient transfection of a wild-type *p53* gene triggers resveratrol-induced apoptosis in cancer cells

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Resveratrol (RV) is a promising chemopreventive agent that mediates many cellular targets involved in cancer signaling pathways. *p53* has been suggested to have a role in the anticancer properties of RV; therefore, we investigated RV-induced cytotoxicity in H1299 cells, which carry a partial deletion of the p53 protein-encoding gene. The results were compared to those observed in MCF-7 cells, which constitutively express wild-type *p53*, and cell viability assays revealed that RV reduced both H1299 and MCF-7 cell viability in a dose- and time-dependent manner. However, MCF-7 cells were more sensitive than H1299 cells to RV when exposed to the drug for 24 hour and at concentrations that were higher than 100 μM. RV also increased *p53* protein levels in MCF-7 cells without altering *p53* mRNA, which suggests post-translational modulation of the protein. In these cells, RV-induced cytotoxicity was partially mediated by *p53* and involved the activation of caspases 9 and 7 and PARP cleavage, which are suggestive of apoptosis. In H1299, RV-induced cytotoxicity was less pronounced, and unlike in MCF-7 cells, cell death was not accompanied by caspase activation. These results support the observation in which MCF-7 cells were positively labeled by TUNEL after being exposed to a 100 μM concentration of RV, while H1299 cells, under similar conditions, were not labeled by TUNEL. However, transient transfection of a wild-type *p53*-GFP gene rendered H1299 cells more sensitive to RV and made the cells more responsive to its pro-apoptotic proper-

ties, similar to what was observed for *p53*-positive MCF-7 cells. Our study provides a possible therapeutic strategy for cancer treatment in tumors that are usually less responsive to conventional therapy due to the loss of the normal function of *p53*.

P01-40
SOCS as an ROS sensor making cell-fate decisions on survival and apoptosis in an isoform-specific manner

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Since the discovery as feed-back inhibitors of cytokine signal transduction system, suppressors of cytokine signaling (SOCS) proteins have emerged as multi-functional regulators in diverse biological processes and stress responses including cancer, inflammation, and autoimmunity, which are often modulated by ROS signal induced by oxidative or genotoxic stimuli. We have recently reported that SOCS1 suppresses ROS-mediated T cell apoptosis through the induction of thioredoxin, which not only acts as an ROS scavenger but also protects protein tyrosine phosphatases from the ROS-induced inactivation by direct molecular interaction upon oxidative stress. Such protective function of SOCS1 is observed upon oxidant treatments or TNF-α stimulation but not upon Fas receptor ligation of T cells. SOCS1, by contrast, promoted Fas–mediated T cell apoptosis through down-regulation of caspase 8 inhibitor c-Flip and mitochondrial anti-apoptotic factor Bfl-1. While ROS signaling for NF-κB activation rescues T cells from Fas-mediated apoptosis by up-regulating survival genes, SOCS1 induced NF-κB ubiqitination, degradation and transcriptional activation, thereby down-regulating NF-κB target gene expression critical for cell survival. Thus, SOCS appears to act as an ROS sensor to suppress ROS signaling in T cells in two different systems. Under high oxidative stress condition, the ROS scavenging function of SOCS1 attenuates ROS-induced stress and cell death, while under low ROS level induced as a defense to the death receptor signal, SOCS1 counteracts ROS-activated survival pathway resulting in the promotion of cell death. The isoform-specificity of SOCS in these responses is also presented.

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P01-41
Proapoptotic and antitumor activity of lactaptin

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Lactaptin, a 8.6 kDa human milk-derived protein, induces apoptosis in cultured tumor cells. Recombinant analog of lactaptin RL2 was constructed and expressed in *E. coli*. RL2 activated caspases 8, 9 and selectively induced apoptosis only in cultured human tumor cells but not in non-transformed cells. Using JC1 and $DiOC_6$ we have shown RL2 induced dissipation of mitochondrial membrane potential in human tumor cells. Using the conjugate of RL2 with rhodamine we have shown RL2 easily penetrated into tumor and non-malignant cells.

Mice hepatocarcinoma A-1 (HA-1) was also sensitive to RL2 *in vitro*. We demonstrated that RL2 induced phosphatidylserine exposure on the HA-1 plasma membrane surface and dissipation of mitochondrial membrane potential. Tumor necrosis factor (TNF) is capable of inducing apoptosis in tumor cells and exhibits strong antitumor activity against several human cancers. Pilot study indicated that RL2 efficiently inhibited tumor growth in mice. The solid- and ascites-producing tumors inhibitory effect of RL2 was compared with that of TNFα. It was demonstrated that repetitive injections of RL2 (50 mg/kg) effectively inhibited HA-1 solid tumor transplant growth whereas (TNF)-α repetitive injections (10^5ME) didn't delay tumor growth with a significant difference from control.

In conclusions, the present study suggests that recombinant analog of lactaptin can be effective as an anticancer therapeutic.

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P01-42
Resistance to apoptosis in endometrial cancer cells

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Apoptosis resistance is a hallmark of cancer. Tumoral cells can develop mechanisms of apoptosis resistance to different apoptotic stimuli. In a first part of our studies, we have investigated the mechanisms that regulate apoptotic cell death by the extrinsic pathway in endometrial cancer cells, with special focus on apoptosis triggered by TRAIL. We first demonstrated that endometrial carcinoma cells are resistant to TRAIL-induced apoptosis due to elevated FLIP protein levels. We further analyzed the molecular mechanisms involved in the regulation of intracellular levels of FLIP. We demonstrated that FLIP levels can be regulated by different proteins at multiple levels. Inhibition of the protein kinase Casein Kinase 2 (CK2), the kinase suppressor of Ras (KSR1) or B-Raf lead to a downregulation of FLIP protein levels and sensitized endometrial cancer cells to TRAIL-induced apoptosis. However, these proteins use different mechanisms to regulate FLIP protein. Whilst CK2 regulate transcription of FLIP mRNA and FLIP protein degradation, KSR1 and B-Raf control FLIP protein translation. In a second part of our studies, we have analysed the role of epithelial cell polarization in the regulation of apoptosis. Cellular polarity has been shown to act as a potent tumour suppressor mechanism in several tissues. Using a novel three-dimensional culture of endometrial cells, we have shown that TGF-β is a proapoptotic for polarized epithelial cells. However, loss of cells polarity switches TGFβ-induced apoptosis epithelial cells to an epithelial mesenchymal transition process. These results suggest that proper cell polarization and establishment of cell-to-cell and cell-to-matrix contacts can be important factors in the regulation of apoptotic process.

P02 – Biochemical Education

P02-1
Analysis of the R230C polymorphism associated with increased obesity and diabetes mellitus type II susceptibility for Biochemistry and Genetic Laboratory Courses

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We have devised and implemented a protocol for an upper division undergraduate laboratory, based on the amplification and analysis of an *ABCA1* gene polymorphism associated with a higher susceptibility to develop obesity and diabetes mellitus type II in students of the FES Zaragoza, UNAM.

First, students read and firm an informed consent about the study. Then collected a drop of peripheral blood cells using a sterile sting in FTA cards to extract genomic DNA and another blood drop was used to measure glucose levels using an electronic glucometer. Students were weighed and measurement of high was also recorded. Samples were then collected by the instructor who assign them a clue and return to students with a different identification number, in such a way that students do not know who the owner of DNA sample is. The *ABCA1* region where the R230C is located was PCR amplified using circles of FTA cards, and the PCR products were digested with the Bsp119I enzyme, to detect the R230C polymorphism. Polyacrylamide gel electrophoresis was used to resolve the PCR products, and the results were statistically analyzed in the context of human population genetics, obesity and diabetes susceptibility. This module enables students to use materials and methods that are routinely used by scientific researchers to analyse polymorphisms in laboratory exercises in the growing field of genomic science and education.

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P02-2
Continuing education courses for science teachers in the Sao Paulo State, Brazil: a positive impact in classrooms

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The rapid advances in many research areas require concomitant actions for education on the new knowledge generated. Brazilian researches funding agencies have invested in the establishment of research centers which also develop the areas of technology transfer and education and dissemination. From these initiatives emerged the education and science dissemination activities of the CBME and INBEQMEDi, in São Carlos (SP, Brazil). One of our activities was the continuing education courses offered to science teachers, in partnership with the SEE-SP. Emphasis was given to methodologies used in the fields of structural molecular biology and biotechnology and to propose appropriate ways to make the teaching and learning of these topics more enjoyable for students. Courses were offered to 123 teachers of SEE-SP in 2011-2012. Each course (30 hour each) included three videoconferences, two face-to-face meetings with activities using handling models of DNA/protein (kit DNA-mRNA-Protein, developed by us), a lecture about DNA/protein engineering and biotechnology and educational strategy for the teaching of related issues. Teachers evaluated the courses positively because it provided autonomy, confidence and criticism on their own practice, since they had the responsibility in building their teaching strategies as well as evaluating it with their students. The impact in the classroom was made by comparative analysis between questions answered by about 300 students (aged 14–17), before and after the activities proposed by teachers (questions: what the importance of the DNA, where is DNA in cell or do you eat DNA?). The increase in the number of students that answered correctly the questions were: importance of DNA in the transmission of characteristics (from 25 to 95%); DNA is in the cell nucleus (18–97%); I eat DNA (35–100%). About 55% of the students associated DNA with some kind of technological application.

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P02-3
Connecting scientists and students: personal narrative breaks down stereotypes and builds understanding

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In an era where science student enrolments are declining and science literacy is falling Australia-wide, it is crucial to develop ways for students and scientists to connect. Students need to know that science is an intensely human endeavor, and scientists need mechanisms to bring that truth to the community at large. We have developed a podcast program called 'Free Energy'. We use a personal narrative approach to interviewing scientists about their life and work. We ask second-year science students to write a personal reflection about one interview as an on-course assessment item. In our first year of implementation (2011) we recorded ten podcasts and obtained 478 students' reflections; we performed inductive analysis on 456. Students described their dreams and hopes for the future. They repeatedly made deeply personal confessions that, surprisingly, revealed fear, insecurity, and confusion about entering science careers. Students told us how surprised they were to discover from the podcasts that scientists 'are just normal people'! Many said that the podcasts had given them new hope about their future in science. The students' voices provide us with extraordinary insight into their needs, strengths, and aspirations. This novel perspective has prompted profound changes in the way our school addresses student recruitment, pastoral care, and opportunities to build connections between students and teaching and research faculty. I will present excerpts from the podcasts and the reflections, as well as a full analysis of the students' writings and the implications their comments have for science education in Australia and beyond.

P02-4
Rethinking the textbook: a marriage between printed and online interactive contents

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This communication describes design decisions for a 2nd edition of a molecular biology book. One of the lead ideas has been to

transfer part of the content to a digital format that allows interaction. The design is not of an 'electronic supplement' or 'companion website' in the sense of extra or complementary materials. Rather, decision has been to remove from the printed version those items that may be offered with higher efficiency and ease of comprehension using other media. This purports to make the more advantageous digital format the primary source of such information. The result is hence an integral resource where neither the paper nor the digital material are self-sufficient, but they intertwine.

The major aims are to ease understanding of the topics, by using richer media, and to allow the readers, by interaction, to become active in the elaboration of their own knowledge.

The online formats are diverse. There are 3D interactive molecular models to appreciate the structure of biomolecules and their interactions. For processes dynamic in space or time, animations are used. Video clips illustrate some concepts difficult to be drawn. Finally, all modules integrate media and the content is responsive to reader's action, to enrich the process of understanding and learning.

Integration, interactivity, modularity of the contents pretend a flexible and dynamic work that adapts to diverse interests and needs, depth or focus, as well as a personalised training experience. We feel that offering not just reference but material to work and interact with may fit well into the current educational trend, exemplified by the Bologna Process for the European Higher Education Area, where the emphasis is put on the student's personal work and the acquisition of abilities.

P02-5
Film as an educational resource for teaching and learning biochemistry: 'Lorenzo's oil'

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The new organization of university education should focus on helping students *acquire competencies* which provide them with skills such as: critical thinking, problem solving, and an understanding of self-learning tools that afford them greater autonomy.

Aim: To introduce film as an educational tool to be used in the teaching and learning of biochemistry and which allows students to acquire the corresponding basic, general and specific competencies set out in the Spanish Qualifications Framework for Higher Education (MECES) and Royal Decree 1393/2007.

Methodology: The film 'Lorenzo's Oil', which is based on a true story, deals with different subjects that are covered in the degree curriculums of Pharmacy, Medicine, etc., with Biochemistry the true driving force behind this film. The methodology includes the following steps: (i) Explanation in class of the related curriculum items, (ii) Introduction to the film: the plot and things to consider when watching the film, (iii) Screening of the film in class, the teacher previously provides the student with a questionnaire, (iv) Responses to the questionnaire are provided through the University Virtual Campus website, (v) Group discussion and conclusions on the social, scientific or ethical issues dealt with in the film.

Results and conclusions: Broad participation and achievement in all phases of the process while showing that film as a teaching resource: improves knowledge retention and learning skills, encourages critical thinking and the creative ability to formulate and present arguments or solve problems, stimulates awareness and interest in research with the understanding that the scientific method leads to the self-learning of new knowledge. In short, film can be a good teaching tool for promoting students' acquisition of important competencies. PIMCD 2011-12 no 405.

P03 – Biochemistry and Molecular Biology of Plants

P03-1
C2-domain protein DEPOLL is required for intine development in *Arabidopsis* pollen

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Developmental processes responsible for the proper male gametophyte formation are one of the most critical steps in plant reproduction. Here we present the functional analysis of the At1g70790 gene (DEPOLL). This gene was originally annotated as transcription factor with C2H2-type zinc finger domain. However, in-silico analyses made this presumption unlikely. We confirmed only the C2 domain in N-terminal region. The mature pollen of depoll plants exhibits the plentiful phenotypic defects including increased number of dead pollen grains. The electron microscopy revealed further defects in the intine ultrastructure and organisation. In depoll pollen, intine was greatly reduced and often nearly missing, passing to granular structure leaving only aperture regions unaffected. Accordingly, the depoll mutant showed no changes in pollen germination. The microarray experiment led to the identification of several gene families affected in mature pollen by depoll mutation including genes related to membrane transport on the base their ability to facilitate *in vitro* transfer of phospholipids between membranes (lipid transfer proteins) and cell wall metabolism (invertase/pectin methylesterase inhibitor family proteins, polygalacturonase family proteins, xyloglucan endotransglucosylase/hydrolases family proteins). We consider DEPOLL function in proper intine and/or plasma membrane structure.

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P03-2
A possible role for auxin in chloroplast movement

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The role of auxin in plants and the functioning of the plant cytoskeleton have both been widely studied for many years. Recently, more and more links between the cytoskeleton and auxin are emerging. For example, both of these components take part in establishing and maintaining cell polarity. Auxin is also known to play a major role in phototropism, a process controlled by blue light via phototropins. On the other hand, both phototropins and the actin cytoskeleton are key elements in the process of chloroplast movement. These connections between auxin and blue light signaling pathways point to probable links between auxin and chloroplast movements.

In experiments performed on *Nicotiana tabacum* and *Tradescantia albiflora* we observed a decrease of chloroplast movement velocity and amplitude after shoot decapitation. The application of exogenous auxin to decapitated shoots allows to overcome or diminish this inhibitory effect. This suggests that auxin produced by the shoot apex influences the process of chloroplast movement. The result of decapitation is not prominent and points to a modulatory activity of auxin rather than a direct involvement in the movement mechanism. The observed effects of shoot decapitation do not seem to be connected with a response to wounding, since the cutting of leaves does not influence chloroplast movements.

P03-3
Functional expression of the triterpenoid biosynthesis tailoring enzyme on enzymatic modification of sterol derivatives

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Triterpene saponins constitute a family of compounds with a triterpene aglycone and sugars structures. Recent researches showed that the triterpene saponins exhibit diverse biological properties including antiinflammatory, antifungal, antimicrobial, antiparasitic, and antitumor activities. Biosynthetic studies suggested that the triterpene saponin structure diversity is generated by (i) cyclization of oxidosqualene to various triterpene skeletons, (ii) hydroxylation or oxidation at multiple positions, and (iii) glycosylation to add sugar moieties through an ether or ester glycoside linkage. We have applied genetic engineering coupled with product characterization to elucidate the structure–function-reaction mechanism relationships of the *Saccharomyces cerevisiae* oxidosqualene-lanosterol cyclase-catalyzed (ERG7) cyclization/rearrangement reaction. Numerous truncated or altered deprotonation products were isolated from the ERG7 mutants. Modification of lanosterol-type core structures with tailored enzymes holds great potential both in increasing structure diversity and in enhancing biological activity. In this study, we express three hydroxylases for *in vivo* and *in vitro* enzymatic modification of sterol derivatives to further increase structural and biological activity diversity. The enzyme-substrate relationships on the catalytic and structural diversity of the ERG7-hydroxylase will be discussed.

P03-4
Antioxidant and antimicrobial activities of walnut *(Juglans regia* L.*)* leaves

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Juglans regia L., the royal species from family Juglandaceae, has been used in traditional medicines from ancient times. Walnut leaves are considered to be a source of healthcare compounds and have been intensively used in traditional medicine for the treatment of venous insufficiency, hemorrhoids, hypoglycemia, diarrhea, and fungal or microbial infections. Antimicrobial and antioxidant activities were determined by using leave samples of *Juglans regia* L. leaves collected from Corum/Turkey and environment in this study. Chloroform and ethanol extracts of walnut leaves was examined for antioxidant and antimicrobial activities. Antioxidant activities were detected spectrophotometricaly by using Erel (2004) methods. Antimicrobial activities of *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 strains evaluated by disc diffusion method. On all microorganisms of walnut

leave extracts inhibition effects were observed in different rates. The most sensitive strain *Staphylococcus aureus* ATCC 25923 (13.5 ± 2.0 mm) and also the most resistance strain *Escherichia coli* ATCC 25922 (6.25 ± 1.0 mm) were determinated in antimicrobial activity studies. Antioxidant activities of walnut leaves were observed in antioxidant activity studies. And also, antioxidant activities of ethanol extract were higher than chloroform extract of walnut leaves.

P03-5
A thermoluminescence study of photosystem II back electron transfer reactions in *Lotus japonicus*

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Thermoluminescence (TL) emission technique has been used to study '*in vivo*' the recombination reactions of photosystem II in leaves of *Lotus (*L.*) japonicus* plants grown in atmosphere rich in CO_2 [0.7% (v/v)]. Excitation of dark-adapted leaf segments at 1°C with a series of single turn-over flashes induced the appearance of complex TL glow curves. The emission curves obtained after two flashes produced a maximal overall emission and could be well simulated by two decomposition components. We assigned the first component to the B-band originating from the recombination reaction of $S_2/_3Q_B^-$ charge pair and the second one to the AG-band due to recombination of $S_2/S_3Q_BX^-$ charge pairs, being X an unknown reductant present in the stroma. Signal contributions and t_{max} of the two bands were estimated for plants both growing in atmosphere rich in CO_2 (B-band, 32% and 25°C; AG-band, 68% and 45°C) and in air (B-band, 46% and 30°C; AG-band, 54% and 45°C). These results show a significant decrease in AG/B bands intensities ratio for plants growing under air as compared to the high CO_2 conditions (from 1.1 to 2.1). High-temperature TL (HTL) emissions associated to lipids peroxidation were also recorded from 10°C to 160°C without light excitation. Leaves of plants grown under air or high CO_2 showed a HTL2-band appearing at 125 and 135°C, respectively. These results show a significant decrease in the HTL2-band intensity for plants growing under air as compared to the high CO_2 conditions. The information obtained by TL on the changes induced in the metabolism of *L. japonicus* by CO_2 will be discussed.

P03-6
Euphorbia characias latex enzymes

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Euphorbia characias is a shrub, belonging to the Euphorbiaceae family, occurring in various habitats in vast areas of the Mediterranean basin. Characteristic of all Euphorbiaceae is the presence of laticifers, specialized elongated cells or vessel-like series of cells, that permeate various aerial tissues of plant. Latex, an often milky variously colored sap, constitutes the cytoplamic content of laticifers. It is generally agred that the ecological role of the latex is to deter insect herbivory and possibly discourage foraging by higher animals.

We discovered the coexistence of multiple enzymatic activities within the latex-driving system of *Euphorbia characias*. Two main players have been characterized in this experimental model, namely an amine oxidase (ELAO), a copper/quinone-containing enzyme catalyzing the oxidative deamination of diamines and polyamines to aldehyde and ammonia, concomitantly with a two-electron reduction of dioxygen to hydrogen peroxide, and a calcium/calmodulin-regulated class III secreted peroxidase (ELP), probably involved in the homeostasis of hydrogen peroxide. These two proteins have been purified and characterized in our laboratory and the respective genes have been sequenced. Moreover, we discovered that catalase and antiquitin genes are expressed in *Euphorbia characias* latex and it permits to sketch a more detailed map of some of the multi-enzymatic interactions that could potentially take place in this unusual environment.

Finally, a pyrophosphatase/phosphodiesterase (ELNPP) and a purple acid phosphatase (ELPAP), purified in the *Euphorbia* latex, seem to be related in the metabolism of this plant. ELNPP can produce phosphomonoesters from diesters of phosphoric acid, and these monoesters can be well hydrolyzed by ELPAP taking to hypothesize an joined action of these important enzymes on the metabolism of nucleotides.

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P03-7
Nitric oxide regulation of stem cell niche homeostasis through auxin crosstalk

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Nitric oxide (NO) is a reactive nitrogen species considered also a signalling molecule involved in a variety of physiological processes during plant growth and development. Our recent research has uncovered a role of NO on primary root growth in Arabidopsis (1, 2). At the cellular level, NO accumulation affects cell meristem size and number and alters auxin transport decreasing the auxin efflux PIN1 abundance. Careful examination by DAF-2DA analysis of 5-d-old WT roots reveals that NO is additionally accumulated in the stem cell niche, composed of an organizing centre (QC), which maintain stem cell identity in the neighbouring population of cells. In order to elucidate the specific role of this molecule in the stem cell niche, we characterized the phenotype of different mutants involved in NO-synthesis or metabolism (*cue1*; *atnoa1*; *nia1nia2*; *atnoa1nia1nia2*) in these cell types. Our results suggest that differentiation of columella stem cell (CSC) daughter into columella cells (CCs) is significantly promoted when the NO source is reduced, either in NO-deficient mutants or using a chemical biosynthesis inhibitor, L-NMMA. Since auxin plays a main role in QC/initial/columella cell organization, we decided to analyze whether the abnormal phenotypes present in these NO-related mutants were related to modifications in auxin metabolism, transport and/or signalling and the putative genetic interactions between both pathways.

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P03r-8
Different approaches for the isolation of efficient promoters to direct the expression of transgenes in microalgae

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Despite the biotechnological interest of microalgae, no robust and stable methods for genetic transformation of most microalgal strains exist. The scanty and disperse data about the ability of heterologous promoters to drive the expression of transgenes in microalgae, and the use of different transformation methods, DNA quantities and reporter genes in the existing studies, makes very difficult a real comparison of their efficiency. In the present work, using *Chlamydomonas reinhardtii* as a host, we have evaluated the efficiency of three heterologous promoters, the Cauliflower Mosaic virus 35S (*CaMV35S*), the *Agrobacterium* nopaline synthase (*nos*) and *Haematococcus* phytoene desaturase (*Hppds*). The promoters were fused to the paromomycin-conferring resistance aminoglycoside 3'-phosphotransferase (*aphVIII*) gene form *Streptomyces rimosus*, and *Chlamydomonas reinhardtii* was transformed by glass beads agitation method. The transformation efficiency, and the *aphVIII* transcript and protein levels were evaluated in a series of transformants for each promoter. The quimeric promoter *hsp70A*/*rbcS2* and the promoter-less *aphVIII* marker gene were used for comparison. Furthermore, using the model chlorophyte *Chlamydomonas reinhardtii* and the paromomycin resistant a*phVIII* gene as a marker, we have demonstrated that random insertion of the promoterless marker gene and subsequent isolation of the most robust transformants allows the identification of new strong promoter sequences in microalgae. The ultimate objective of these studies is the selection of the best promoters to establish transformation methods in other chlorophytes which are refractory to genetic manipulation or are transformed with low efficiency.

P03-9
Isolation and characterization of the zeaxanthin epoxidase gene of *Chlorella zofingiensis* using *Chlamydomonas* mutants

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In the xanthophylls cycle, the violaxanthin de-epoxidase and zeaxanthin epoxidase catalyze the interconversion between violaxanthin and zeaxanthin in both plants and green algae. These xanthophylls participate in maintaining a balance between the capture, the use of light and the cell protection from the excess of excitation energy. In fact, the dissipation of excess of excitation energy mediated by zeaxanthin is a key process in the photosynthetic systems. In this work, the zeaxanthin epoxidase from *Chlorella zofingiensis* gene was isolated (*Czzep*), encoding a polypeptide of 596 amino acids and, being present in a single copy in the *C. zofingiensis* genome, as shown by Southern blot analysis. *Czzep* qPCR analysis done under high light irradiance showed that the transcripts of this gene are enhanced after zeaxanthin formation. In addition, the functionality of this gene in the *Chlamydomonas* mutant *npq2*, which lacks ZEP activity and therefore exhibits low quantum efficiency (Fv/Fm) and accumulates zeaxanthin in all conditions, was performed. The ZEP gene was adequately inserted in the pSI105 vector and expressed in the mutant *npq2*. The positive transformants were able to efficiently transform zeaxanthin into violaxanthin and also the maximum quantum efficiency (Fv/Fm) of the wild type was restored. These results show, once more, that *Chlamydomonas* can be an efficient tool for the functional characterization of heterologous genes.

P03-10
Proteome analysis of cell nuclei enriched subcellular fraction of apple *(Malus × domestica Borkh.)*

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Proteome analysis has been an important source of information for system biology analysis of complex molecular mechanisms involved in plant development, productivity and response to environmental stimuli. However, proteome of entire plant cell presents high demands on dynamic range and sensitivity of protein and analysis procedures. The problems encountered due to the complexity of sample could be overcome by application of subcellular fractionation in preparation of samples for proteomics analysis. Information on nuclear proteome of plants of the *Rosaceae* family remains vague. Apple, the most economically significant plant of the *Rosaceae* family, is also an ideal model for genomic studies on woody plants of the *Rosaceae* family due to availability of genomic information. In this study, we developed a procedure for apple cell nuclear protein enriched fraction preparation and 2D gel electrophoresis based analysis. Apple cell nuclei isolation conditions were established and optimised based on previously published results of the studies on plant nuclei preparation. Efficient cell breakage of apple leave tissue was ensured by grinding in liquid nitrogen, and filtration followed by differential centrifugation led to separation of organellar fractions. For further enrichment of nuclei, a differential lysis of organelles was employed and concentration of detergent was optimized. The nuclei were separated from other organelles by equilibrium centrifugation on combined sucrose and percoll gradient. The enrichment of nuclear proteins and contamination with non-nuclear proteins was analysed using specific antibodies. Isolated protein samples were subjected to 2D gel electrophoresis. Specificity and sensitivity of the method was assessed by comparing the results obtained using nuclear and total cell protein fractions.

P03-11
Covalent and noncovalent complexes of soybean storage proteins, beta-conglycinin, P34 and glycinin in cotyledon cell

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Beta-conglycinin, one of the major soybean (*Glycine max*) seed storage proteins, is folded and assembled into trimers in the endoplasmic reticulum and accumulated into protein storage vacuoles (PSV). Beta-conglycinin is believed to accumulate in the PSV in the form of a noncovalently associated trimer, based on experiments performed in the presence of sulfhydryl reductant such as 2-mercaptoethanol. In this study, we extracted soybean proteins from the cotyledons of immature seeds or dry beans under nonreducing conditions to prevent oxidation of thiol

groups and reduction or exchange of disulfide bonds. As a result, we found that approximately half of the alfa' and alfa subunit of beta-conglycinin were disulfide-linked, together or with P34, prior to N-terminal propeptide processing. Sedimentation velocity experiments, size exclusion chromatography, and two-dimensional polyacrylamide gel electrophoresis (PAGE) analysis, with Blue native-PAGE followed by sodium dodecyl sulfate-PAGE, indicated that the beta-conglycinin complexes containing the disulfide-linked alfa'/alfa subunits were complexes of dodecamer. The alfa' or alfa subunits, when disulfide-linked with P34, were mostly present in hexamer. Our results suggest that disulfide bonds are formed between alfa'/alfa subunits residing in different beta-conglycinin hexamers, but the binding of P34 to alfa' and alfa subunits reduces the linkage between beta-conglycinin hexamers. Finally, we found a subset of other major storage protein, glycinin existed as noncovalently associated complexes with beta-conglycinin by two-dimensional PAGE analysis. Our data suggest that the associations of pro alfa' or pro alfa subunits with P34 or glycinin may play an important role in the transport of P34 and glycinin to the PSV via Golgi bodies.

P03-12
Expression profiling of glutathione transferase (*Gst1*) gene in maize seedlings infested by the bird cherry-oat aphid *(Rhopalosiphum padi* L.)

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The purpose of performed analyses was to evaluate the impact of bird cherry-oat aphid (*Rhopalosiphum padi* L.) feeding on the transcriptional activity of glutathione transferase (*Gst1*) gene within maize seedlings. Furthermore, the selected entomological parameters (length of prerepro-ductive period and daily fecundity) were recorded in order to assess the average time of generation development (T) and the intrinsic rate of natural increase (r_m) of the aphid population on *Zea mays* plants.

The bioassays were conducted in a growth chamber at four levels of aphid infestation (5, 10, 20 and 40 individuals per seedling). Transcriptional responses of *Gst1* gene in aphid-infested maize seedlings were investigated at 1, 2, 4, 8, 24 and 48 hour post infestation (hpi). The qRT-PCR technique was used to quantify the relative expression of the targeted gene in maize tissues (GAPDH, glyceraldehyde 3-phosphate dehydrogenase gene was used as a reference control).

Expression profiles of the *Gst1* gene within *R. padi*-stressed maize seedlings showed differential transcript accumulation in dependence on the number of aphid individuals and duration of exposure to the examined biotic stressor. The expression levels of analysed gene in maize tissues were gradually elevated during the early phases of infestation (1, 2 and 4 hpi) when compared to the aphid-free control. Importantly, the most substantial up-regulation of *Gst1* gene in stressed *Z. mays* seedlings was reached at 8 hour of aphid infestation. The presented results evidence the involvement of cytosolic GSTI enzyme in overcoming detrimental imbalance in the redox status as a result of the aphid-triggered oxidative burst in maize seedlings.

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P03-13
The *N. tabacum RIPA1* gene encodes for a novel pollen tube protein involved in Rac5 GTPase dependent control of polarized cell growth

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Polarization of eukaryotic cells through signaling of Rho-GTPases is essential for morphogenesis, division, and motility of unicellular organisms and plays a key role in differentiation and development of various cell types in metazoa. Polarized cell growth in pollen tubes of Nicotiana tabacum is controlled by the Nt-Rac5 GTPase that accumulates in the tube tip and belongs to a plant specific subfamily of Rho-GTPases. In a yeast two hybrid screen for unknown effectors of Nt-Rac5 we identified the unspecified protein Nt-Ripa1. Here we functionally characterize the Ripa1 protein that is exclusively expressed in growing pollen tubes. Ripa1 is a 600aa protein that comprises an N-terminal hydrophobic region predicted as trans membrane domain, as well as a C-terminal domain of unknown function (DUF593). Transient over expression of Ripa1 inhibits tobacco pollen tube growth and interferes with membrane trafficking and secretion. Localization studies with YFP-Ripa1 constructs in *N. tabacum* pollen tubes reveal that Ripa1 resides at the apex of the growing tip, sub-apical of the vesicle-accumulation zone. Our pull down data identify Nt-Ripa1 to be a physical interactor of Nt-Rac5. We performed a deletion analysis of the *RIPA1* gene and show that interaction between Rac5 and Ripa1 is restricted to the N-terminus of Ripa1. Interestingly we could provide evidence that Ripa1 co-localizes with known trans golgi network (TGN) markers sub-apical in the pollen tube tip near the actin ring. Subsequent binding studies could provide evidence that Ripa1 associates with Actin. Analysis of several Ripa1 truncations shows, that DUF593 is responsible for the specific localization pattern of Ripa1 sub-apical of the vesicle accumulation zone. We conclude that Ripa1 associates with post Golgi-organelles and the actin cytoskeleton, hence linking membrane trafficking to polarized cell growth regulated through the Rac5-GTPase.

P03-14
Cyclin D-CDKs during maize seed germination: activity and regulation

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The cell cycle has a determinant role in growing and development of multicellular organisms, including plants. During germination cells restart cell division and activate the cell cycle machinery to generate a new plant. Some of the basic mechanisms that regulate cell cycle is the formation of cyclin-CDK complexes, which determine cell cycle progression. Although mammals and plants share similarities in the regulatory machinery, plants contain specific types of CDKs and cyclins that have no orthologs in mammals. The aim of this work is to study regulatory events of some G1 phase cyclin-CDK complexes during maize germination. We have

determined the levels of cyclins D2;2, D4;2 and D5;3, their interaction with CDKA and CDKB, the phosphorylation levels of CDKs and their kinase activity at different times of maize germination. Our results demonstrate that cyclins D2;2, D4;2 and D5;3 associate not only with CDKA, but also with CDKB. We found fluctuations in the levels of CDKA associated with D-type cyclins along germination. Also, we detected that variations in CDKB-cyclin D complexes differ to those of CDKA-cyclin D complexes, suggesting that cyclins D bind CDKs in a differential way, and thus cyclin D-CDK complexes may regulate different aspects of cell cycle progression. In order to establish if cyclin D-CDK complexes are active, we determined the levels of phosphorylation of CDKs during germination, as well as kinase activity in complexes. Our results indicate that there is a correlation between CDK-cyclin D association, CDK phosphorylation and kinase activity. These results suggest that kinase activity in CDK-cyclin D complexes may be regulated by the CDK phosphorylation state and also that these complexes are regulated differentially along maize germination.

P03-15
Development of eleven polymorphic microsatellite markers for a Myrtaceae species

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Pimenta pseudocaryophyllus (Gomes) Landrum, popularly known as cataia in Brazil, belongs to the Myrtaceae, one of the dominant woody families in the Atlantic Rainforest. In the Vale do Ribeira, São Paulo, Brazil, this species is widely consumed for medicinal purposes as a tea in fighting colds, influenza and fatigue, and as a diuretic; it is also used for inhalations and massages. Currently, its leaves are marketed in large quantities as a flavoring agent of alcoholic drinks. Despite the wide variety of human uses for this plant, little is known about the consequences of the anthropogenic pressure or even about its biology. In order to develop useful information for studies of the genetic diversity and population structure, we isolated and characterized a set of microsatellites from this species. A microsatellite-enriched library was developed, and 15 primer pairs were designed. PCR reactions were performed based on *touchdown* PCR strategy. Twelve of these primer pairs generated consistent patterns of amplification, and were used for polymorphism analysis, that considered a total of 35 individuals belonging to two different natural populations. Of the 12 microsatellite markers, 11 were polymorphic. The number of alleles per locus ranged from 1 to 15. Polymorphism information content values ranged from 0 to 0.869. The observed heterozygosity (H_o) and the expected heterozygosity (H_e) were 0–0.941 and 0–0.903, respectively. Six loci showed a significant deviation from the Hardy–Weinberg Equilibrium. Nine loci showed private alleles when the populations analyzed were compared. These microsatellite markers are the first to be developed for *P. pseudocaryophyllus,* and have great potential for use in genetic studies of wild populations. These studies may increase understanding of its biology and help to detect ongoing consequences of its use, in the context of conservation genetics.

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P03-16
Juglone-triggered alternations in expression of glutathione transferase *(GstI)* gene in maize seedlings

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Juglone (5-hydroxy-1, 4-naphthoquinone) has been isolated from tissues of many plant species belonging to *Juglandaceae* family. It has been hypothesised that this allelochemical functions as a direct oxidant that stimulates generating reactive oxygen species (*ROS*) in acceptor plants. Phytotoxic effects of juglone may lead to suppression the intensity of a broad spectrum of physiological processes in plant organs. Glutathione transferases (GSTs, EC 2.5.1.18) represent an important group of enzymes participating in detoxication of xenobiotics and limiting oxidative damages of cellular biomolecules.

The performed study was aimed at establishing the effect of juglone on levels of glutathione transferase (*GstI*) gene expression in maize seedlings. Additional objectives were to evaluate the impact of this allelocompound on growth and development of primary roots and coleoptiles of *Zea mays* seedlings. Transcriptional activity of *GstI* genein juglone-stressed maize seedlings was monitored at the 4th and 8th day of experiments. The qRT-PCR technique was applied in order to quantify the relative expression of analysedgene in maize tissues.

The allelopathic biotests revealed that 4-day juglone treatment significantly enhanced the amounts of *GstI* mRNA transcripts in *Z. mays* seedlings comparing to control plants. At the 8th day of experiment the transcriptional responses were slightly lower when compared to the shorter one. It was demonstrated that levels of up-regulation of targeted gene, as well as the inhibition of primary roots and coleoptile elongation were proportional to tested juglone concentrations. It is noteworthy that primary roots of maize possessed higher levels of *GstI* gene expression than coleoptiles. Presented results provide the strong molecular evidence that allelopathic effects of juglone on growth and development of the maize seedlings may be associated with induction of the oxidative stress in plant tissues.

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P03-17
Differential expression of metallothionein genes in silver hyperaccumulating ectomycorrhizal fungus *Amanita strobiliformis*

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Mycorrhizal fungi play an important role in the environmental cycling of elements and protection of their host plants against various stress factors. We previously showed that Ag-hyperaccumulating *Amanita strobiliformis* expresses three metallothionein genes, As*MT1a*, *1b* and *1c*, with As*MT1a* mRNA being the dominant transcript. Here we demonstrate that all isomorphic AsMT1 expressed in *Saccharomyces cerevisiae* had the capacity

of to sequester Ag, Cd, Cu and Zn. Comparative transcript analyses showed that Ag and Cu, but not Cd and Zn, strongly induced expression of As*MT1*s in metal-exposed mycelia of *A. strobiliformis*. These analyses also confirmed differential expression of As*MT1*s in a response to Ag. We also report on isolation of transcript encoding AsMT2, which appeared induced solely by Cd. Its predicted peptide sequence is dissimilar to AsMT1s, but 65% identical to that of PiMT of *Paxillus involutus*. In order to get insight at the genetic level concerning differential expression of As*MT1*s and As*MT2*, the entire genes including promoter regions were amplified. All As*MT*s contained two introns, metal response elements (MRE) and oxidative stress-response factor AP1-binding elements were identified within individual promoters. Promoter region of As*MT2* further contained binding sites of heat shock factor (HSF). The TATA/CAAT boxes were found duplicated in As*MT1a* promoter, but not in promoters of other As*MT*s. Possible impact of differences in distribution and abundance of potential control elements on differential expression of As*MT*s will be discussed.

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P03-18
Lavandula angustifolia oil from Spain: aromatic profile by enantioselective gas chromatography-mass spectrometry

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The separation and determination of the chemical composition of the *Lavandula angustifolia* oil from Murcia was performed by Fast Gas Chromatography with Mass Spectrometry Detection (FGC-MSD), whereas the enantiomeric separation and determination was made by Enantioselective Gas Chromatography with Mass Spectrometry Detection (EsGC-MSD). FGC used a non polar fast column (SLB-5ms) 15 m × 0.1 mm × 0.1 μm with hydrogen as a carrier gas. EsGC used a chiral column Chiraldex B-DM 30 m × 0.25 mm × 0.12 μm with hydrogen as a carrier gas. The MSD contained a 70 eV electronic impact ionization source, and a sigle quadrupole analyzer. Calibration straights with ten concentrations and triplicate chromatograms, were carried out for each volatile biomolecule.

The chromatographic results showed that the *Lavandula angustifolia* oil from Murcia is especially rich in some components like Linalool and Linalyl acetate. The components, regarded in the ISO normative, depicted these results of concentration in mM, (enantiomer proportions between brackets): Limonene 7.37, 3-Octanone 46.66, Eucalyptol 78.18, Camphor 97.58 (+51.7%, −48.3%), Z-Ocimene 111.38, α-Terpineol 175.46 (+71.5%, −28.5%), Linalyl acetate 2489.31, Linalool 5042.22 (+2.4%, −97.6%).

The chiral results depict a distribution of positive and negative enantiomers produced by *Lavandula angustifolia*, where Linalool is mainly negative enantiomer, Camphor is almost racemic mixture and α-Terpineol has a dominant positive enantiomer.

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P03-19
The significance of flax fibre pectin in the extracellular matrix remodelling of wound healing process

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Pectin is a structurally complex family of polysaccharides and as the plant cell wall component is responsible for a plant development, growth and the defence for the pathogen infection. Pectin is underestimated natural resource with multi-aspect applications in a food, pharmaceutical and medical industry. New research revealed the possible role of pectin in the extracellular matrix remodelling suggesting its participation in the wound healing process.

Our recent data show a positive effect of the dressing from flax fibre on the healing of wounds of different aetiology due to the adequate structure of flax fibre and the presence of active components: hydrophilic (phenolic acids) and hydrophobic (terpenophenols) components with ROS scavenging activity and anti-inflammatory properties.

The aim of this study was a verification of the influence of flax pectin on the extracellular matrix remodelling process. Flax fibre enriched in pectin was obtained from the transgenic flax overexpressing β-glucanase, PR protein hydrolysing β-glucan, the major component of the fungi cell wall and contributing to the plant defence response. Three fraction of pectin (WSF – water soluble fraction, CSF – CDTA soluble fraction and NSF – Na_2CO_3 soluble fraction) from control and the transgenic plants were analysed in order to check how the genetic modification changes the structure and composition of pectin. The uronic acids content, the monosaccharides compositions and the content of phenolic acids connected with pectin were determined. The antioxidant potential was checked by using the TBARS test. The expression of selected genes involved in extracellular matrix remodelling of normal human dermal fibroblast cell after treatment of flax fibre pectin was analysed by real time PCR.

P03-20
Reducing pectin level in flax plants: new quality of flax fibre

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Flax is the dominant fibrous plant cultivated in a temperate zone and its fibre is priced because of strength, absorptivity and biological activity, which is by far the greatest advantage over cotton fibres. Apart from pectin, a flax fibre cell wall is built from cellulose, hemicellulose and lignin. Located on the outer surface of the cell wall pectin glues fibre cells together.

Quality of fibre highly depends on retting time, the longer the exposition to atmosphere, the worse fiber's hue, texture and mechanical resistance. While possessing fibre from a straw pectin is the target component for microorganisms, thus pectin amount determines retting time and by extension fibre quality.

The subject of the study was to investigate composition and structure of a cell wall in the fibre possesed from flax plants with reduced pectin level. Further, mechanical properties of the newly obtained flax fibre was verified.

Reducing pectin level in flax plants was achieved by overexpressing fungal gene (*Aspergillus aculeatus*) encoding enzyme, polygalactouronase (PGI) or rhamnogalactouronase (RHA), degradating pectic sugars. To obtain fibre and evaluate how the modification affect fibre cell wall, field trial was conducted, one line per each modification was chosen. For both lines biochemical analysis of the fibre cell wall revealed strong, over 30% decrease in pectin level which was followed by slight decrease in lignin amount and compensate by increase in cellulose level, as compared to the control fibre. Infra-red spectroscopy confirmed biochemical analysis and showed changed conformation of chemical bonds within cell wall. SEM micrograpghs and Instron tensile test revealed much better quality and mechanical properties of fibre form transgenic lines.

P03-21
Sulfide prevents early senescence and autophagy induction caused by DES1 deletion in *Arabidopsis thaliana*

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Arabidopsis thaliana, DES1 is the only identified L-cysteine desulfhydrase located in the cytosol that catalyzes the desulfuration of L-cysteine to sulfide plus ammonia and pyruvate. A role of DES1 in plant metabolism is evidenced by the phenotypes of the T-DNA insertion mutants *des1-1* and *des1-2*. Mutation of the *DES1* gene leads to premature leaf senescence, as demonstrated by the increased expression of senescence-associated genes and transcription factors and the detection of senescence-associated vacuoles in mesophyll protoplasts. Additionally, DES1 deficiency promotes the accumulation and lipidation of the ATG8 protein, which is associated with the induction of autophagy. Besides, when GFP-ATG8a fusion protein is introduced into the *des1-1* plants, autophagosome-like punctuate structures are observed in leaf cytoplasm by confocal microscopy. In addition, the transcriptional profile of the *des1-1* mutant strongly correlates with its premature senescence and autophagy-induction phenotypes.

Mutations of the *DES1* gene impede H_2S generation in the *Arabidopsis* cytosol. Restoring the capacity of H_2S generation, through exogenous sources or by genetic complementation, eliminates the phenotypic differences of the *des1* mutants from wild type plants. The mutation of the *DES1* gene also disrupts the production of ammonium from cysteine in the cytosol. However, exogenous ammonium does not have the same effect on autophagy as exogenous sulfide. Therefore, cysteine-generated sulfide in the cytosol negatively regulates leaf senescence and autophagy and modulates the transcriptional profile of *Arabidopsis*. Our results provide insight into the role of sulfide metabolically generated in the cytosol as a signaling molecule.

P03r-22
Perception of copper in the cyanobacterium *Synechocystis* sp. PCC 6803

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Copper is an element required for essential biological processes such as respiration, through the cytochrome oxidase, or in photosynthesis through the electron transfer protein plastocyanin in plants, some algae and cyanobacteria. Copper is also required as metal cofactor on the active sites of different enzymes including oxidases, monooxygenases, dioxygenases and superoxide dismutases. However, if copper is unbound inside the cell, it could be highly, largely due to its ability to catalyze Fenton-like reaction, causing the production of highly reactive hydroxyl radicals that damage biomolecules such as DNA, proteins, and lipids (1). Hence organisms have developed homeostatic mechanisms to tightly regulate its acquisition, sequestration and efflux. We have characterized a two-component system (CopRS) that its essential for copper resistance in *Synechocystis* sp PCC 6803. The CopRS system regulates the expression of a HME-RND efflux system (CopBAC) and its own expression, in response to the presence of copper. Mutants in any of the *cop* genes render cells more sensitive to the presence of copper. To our knowledge this is the first copper resistance system described in cyanobacteria.

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P03-23
Plant wounding influences on neighboring plant immunity

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Many plants release airborne volatile compounds in response to wounding due to pathogenic assault. These compounds serve as plant defenses and are involved in plant signaling. The mechanisms by which these signals promote plant immunity remain elusive. We demonstrate that plant leaf wounding results in the synthesis of a cell wall enzyme, pectin methylesterase (PME), causing the plant to release methanol into the air. Gaseous methanol or vapors from wounded PME-transgenic plants induced resistance to the bacterial pathogen *Ralstonia solanacearum* in the leaves of non-wounded neighboring 'receiver' plants. To investigate the mechanism underlying this phenomenon, we identified the methanol inducible genes (MIGs) in *Nicotiana benthamiana*, most of which fell into the category of defense genes. We selected and isolated the following genes: non-cell-autonomous pathway protein (NCAPP), β-1,3-glucanase (BG), and the previously unidentified MIG-21. We demonstrated that BG, MIG-21 and NCAPP could enhance cell-to-cell communication and *Tobacco mosaic virus* (TMV) RNA accumulation. Moreover, gaseous methanol or vapors from wounded plants increased TMV reproduction in 'receivers'. Thus, methanol emitted by a wounded plant enhances antibacterial resistance as well as cell-to-cell communication that facilitate virus spreading in neighboring plants.

P03-24
Natural rubber and antioxidants in *Euphorbia characias* latex

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We have selected the mediterranean shrub *Euphorbia characias* as an experimental model to study the complexity of plant latex chemistry. Latex is a mixture with diversified composition, that includes alkaloids, terpenoid compounds, polymeric substances such as resins and gums, starch, oil and a large number of proteins and enzymatic activities. The aim of the present study is to contribute to the knowledge of this plant product evaluating the antioxidant properties of extracts of *E. characias* and searching for polymeric substances as natural rubber.

We analyzed different extracts from the latex of *E. characias* and performed a new extraction method (involving the use of trichloacetic acid, TCA) that turned out to be easier, faster and higher reproducible if compared to common extraction methods involving organic solvents like methanol, ethanol, and petroleum ether/methanol.

TCA extract of *E. characias* latex exhibits antioxidant activities determined as total content of free-radical scavenging, polyphenols and total flavonoids. GC-MS analysis confirms the presence of several compound identified as antioxidant molecules.

E. characias latex contains a natural rubber. The optimum rubber extraction is achieved with acetic acid followed by cyclohexane/ethanol treatment. The rubber content was shown to be 14% (w/v) of the *E. characias* latex and the gel content is 2.5% of the rubber weight.

E. characias natural rubber showed a molecular weight of 93000 and M_w/M_n of 2.9. On the basis of ^{1}H NMR, ^{13}C NMR and FT-IR spectroscopy, the structure of this rubber can be identified as *cis*-1,4-polyisoprene.

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P03-25
Identification and characterization of SlCIPK6 phosphorylation targets involved in plant innate immunity

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Plants respond to pathogen attack inducing an immune response, which has two layers. The first layer, called PTI (Pathogen Associated Molecular Patterns [PAMPs]-Triggered Immunity), was traditionally known as basal immunity and is effective against whole species of microbes. The ETI response (Effector-Triggered Immunity) constitutes a second layer of immunity, more intense and powerful, and frequently accompanied by a form of programmed cell death (PCD) in infected cells to limit pathogen proliferation.

Both responses share signal transduction pathways resulting in a sequence of common molecular events, such as the increase in cytosolic Ca^{2+}, the production of reactive oxygen species (ROS) and the activation of mitogen-activated protein kinase (MAPK) cascades.

Previously, two genes involved in PCD associated with ETI were identified in a random Virus Induced Gene Silencing (VIGS) screening in *Nicotiana benthamiana*. Later, we characterized that the proteins encoded by these genes, the calcium sensor *Nb*CBL10 (Calcineurine B-Like protein 10) and the intracellular protein kinase *Nb*CIPK6 (Calcineurine B-Like Interacting Protein Kinase 6), constitute a Ca^{2+} signaling module. To identify phosphorylation targets of CIPK6 in biotic stress, a yeast two hybrid screening was carried out. Here we describe the biochemical and physiological analysis of two of the CIPK6-Interactor Proteins (CIPs) identified, named CIP70 and CIP90. These proteins seem to play an important role in the cellular processes regulated by CBL10/CIPK6 signaling module.

P03m-26
Implication of granule-bound starch synthase (GBSS) in photoperiodic signaling

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Starch is the main carbon reserve in higher plants and constitutes the mayor energy reserve component of some of the most important crops for human consumption. Starch is synthesized by ADP glucose pyrophosphorylase and starch synthase enzymes as *GBSS*. *GBSS* enzyme catalyzes amylose synthesis, the linear component of the glucose polymer in starch. The circadian-regulated gene *CONSTANS* (*CO*) plays a central role in the photoperiodic control of the floral transition. Recently, a *CO* homologue gene present in the genome of the unicellular green alga *Chlamydomonas reinhardtii* (*CrCO*) was identified. *CrCO* has a conserved role in the coordination of processes regulated by photoperiod and the circadian clock (1). To study the rate of conservation of the photoperiodic signaling, starch and hexoses levels were analyzed in different *Arabidopsis thaliana* mutants implied in photoperiod regulation, both before and after blooming. In addition, amylose amount were quantified. *As*GBSS expression is controlled by*CrCO* in *Chlamydomonas reinhardtii* (2), in order to study the same effect in *Arabidopsis thaliana*, we followed the expression of the *GBSS* gene in several mutants in short and long day conditions (3). Moreover, *GBSS* was fused to GFP to study its tisular localization during a 24 hour period. *CO*-overexpressing and *CO* mutant plants were crossed to *GBSS* mutant and their floral phenotype observed. The effect of *CO* on *GBSS* could be part of a new regulatory mechanism connecting photoperiodic signaling with carbon metabolism in plants (4).

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P03r-27
A new redox component in the mechanisms for adaptation of plants to light stress

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When the light energy absorbed by photosystem II (PSII) exceeds its capacity for utilization, the concomitant production of reac-

tive oxygen species may lead to damage of macromolecules and loss of photosynthetic productivity, i. e. photoinhibition. However, plants have evolved strategies to adapt to conditions of excess light. Long-term regulation of the transfer of excitation energy to PSII involves changes in the expression of the genes encoding the light harvesting complex II (LHCII) proteins. In the short-term (min) a process referred to as non-photochemical quenching (NPQ) functions in the dissipation of the absorbed light energy as heat, thus relieving part of the excitation pressure. Perception of excess light may occur through the acidification of the thylakoid lumen and through the action of some photoreceptors, such as chryptochromes (1). It has also been suggested that thioredoxins (Trxs) could be involved in this process, since their redox states depend on the light intensity (Li *et al*., 2008). We have analysed the possible functions of some plastid redox enzymes in the adaptation of plants to light stress both in the long and short terms. To this end, we have studied the responses to high light intensities of *Arabidopsis thaliana* knockout mutants lacking the NADPH thioredoxin reductase C (NTRC), 2-Cys peroxiredoxins or Trx *x* (2). We found that the NTRC knockout mutant displays extremely high levels of NPQ even under moderate light intensity and, in agreement with these data, shows altered levels of the xanthophyll pigments, violaxanthin, antheraxanthin and zeaxanthin. These results suggest that the NTRC enzyme is involved in the redox regulation of the xanthophyll cycle.

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P03-28
Abiotic stress induced differential translation regulation in plants

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Plants, as sessile organisms, have been forced to genetically evolve physiological, biochemical and molecular strategies that allow them to cope with environmental stresses. These complex responses require a deep molecular regulation involving changes at the transcriptional, post-transcriptional, translational and post-translational levels. In the last few years, the variations in the plant transcriptional profiling induced by different abiotic stresses have been broadly characterized. However, although several evidences suggest that the plant protein synthesis is strictly regulated under stress situations, causing (1) a general translation inhibition, even for those messengers that are transcriptionally induced under stress conditions, and (2) the selective translation of some mRNAs generally codifying proteins involved in the abiotic stress response, the individual changes induced at the translational level have been poorly characterized.

The main goal of our research is the characterization of the mechanisms that regulate proteins synthesis in response to stress and the identification of proteins that are preferentially translated in such conditions in the model plant *Arabidopsis thaliana*. We are trying to isolate regulatory proteins that interact with the key components of the translation initiation machinery, mainly with the *cap*-binding factor eIF4E/eIFiso4E, and identify the mRNAs that are specifically associated with polysomes under stress conditions. These studies could contribute to improve the general knowledge on plant protein synthesis regulation and will open new insights on how plants respond to environmental stresses.

P03-29
Flower asymmetry is established by a molecular antagonism involving three MYB-like proteins

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The action of antagonistic proteins on the transcriptional activity of developmental key genetic programmes is an important regulatory mechanism in the generation of different morphologies that characterise multicellular organisms.

In Antirrhinum majus, the dorsoventral asymmetry of the flowers is established through a molecular antagonism involving two MYB-like transcription factors, RADIALIS (RAD), and DIVARICATA (DIV). RAD is expressed dorsally in the floral primordia and promotes dorsal petal identity, whilst DIV is expressed in the whole floral primordia, despite having a phenotypic effect only in more ventral regions of the flower. Genetic and molecular studies have revealed that RAD antagonises the activity of DIV through an indirect interaction with another MYB-like protein identified on a yeast two-hybrid screen using RAD as bait (RIPs, RAD-interacting proteins).

To better understand the role of the RIP proteins in the molecular antagonism that RAD exerts over DIV function, the DNA-binding activity of DIV was analysed on a gel shift assay in the presence of the RIP and RAD proteins. Additionally, the subcellular co-localisation of these three proteins was determined using fluorescent tagged proteins in transient assays in tobacco epidermal cells. The expression pattern of the RIP genes was analysed by RT-PCR and the evolutionary history of these new MYB-like proteins studied by phylogenetic analysis.

Our results suggest that the molecular antagonism that RAD has over DIV is mediated by the RIP proteins. In the ventral domain of the flower DIV and RIPs interact inside the nucleus in order to determine the ventral identity. However, in the dorsal domain, where RAD is present, RAD interacts with the RIPs in the cytoplasm and prevents their nuclear localisation, preventing DIV from interacting with the RIPs, which results in flower dorsal identity. These results along with the fact that the RIP, RAD and DIV proteins are present in such a variety of species including angiosperms without flower asymmetry, suggest that the molecular interactions between the RIP, DIV and RAD may have been implicated in a broader set of functions throughout plant evolution.

P03-30
Ear nitrogen metabolism and maize yield

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The main steps of nitrogen (N) metabolism were characterized in the developing ear of the two maize lines F2 and Io, previously used to investigate the genetic basis of N use efficiency (NUE) in relation to yield, and in the developing ears of glutamine synthetase (GS) deficient mutants (*gln1-3*, *gln1-4* and *gln1-3*/*gln1-4*) exhibiting a reduction in kernel yield. Integrative analysis of physiological and gene expression data indicated that in maize there is genetic and environmental control of N metabolism not only in vegetative source organs but also in reproductive sink organs. Quantitative trait loci (QTLs) for the main steps of N metabolism in the developing ear of maize (*Zea mays* L.) and

their co-localization with QTLs for kernel yield and putative candidate genes were also searched in order to identify chromosomal regions putatively involved in the determination of yield. Glycine and serine metabolism in developing kernels and the cognate genes appeared to be of major importance for kernel production. The importance of kernel glutamine synthesis in the determination of yield was also confirmed. We have extended our investigation using a panel of 19 maize lines currently used for association genetic studies. They are analyzed in order to first determine if there is some genetic variability for NUE marker physiological, molecular markers and traits related to yield. Our objective is to determine if the genetic variability observed for these traits, in a given genetic background can be further used through a statistical approach to improve NUE and yield in a breeding program including commercial hybrids or genetically modified plants production.

P03-31
Glucosyltransferase enzymes from *Crocus sativus* associated with cell division processes

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Glycosyltransferases (GTs) play diverse roles in cellular metabolism by modifying the activities of structural and regulatory metabolites. Three UDP-glucosyltransferase-encoding genes have been isolated from saffron (*Crocus sativus*), UGT85U1, UGT85U2 and UGT85V1, which are closely related to the UGT85 family that includes members associated with cytokinins glucosylations and cell cycle regulation. *Arabidopsis* was used as model system to examine molecular mechanisms underlying developmental effects of UGT85U1. *Arabidopsis* overexpressing UGT85U1 mRNA under the control of the cauliflower mosaic virus (CaMV) 35S promoter exhibited reduced root growth, and increased anchor root development. The timing of root emergence in wild-type and UGT85U1 over-expressing plants was identical, and no differences were observed in leaves or flowering time. The expression levels of several genes related with root development were determined in several transgenic lines, and the role of these GTs in cytokinin metabolism is discussed.

P03-32
Function of flavodiiron proteins in *Anabaena* PCC 7120

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Flavodiiron proteins (FDP) (or A-type flavoproteins), involved in detoxification of O_2 or NO in anaerobic Bacteria and Archaea, were found in all so far sequenced cyanobacterial species. Genome of *Synechocystis* PCC 6803 contains four genes encoding FDPs. The Flv1 and Flv3 proteins function in photoreduction of O_2 directly to water (cyanobacteria-specific type of Mehler reaction) donating electrons to molecular oxygen on the reducing side of Photosystem I. The Flv2 and Flv4 proteins participate in photoprotection of Photosystem II.

Filamentous N_2-fixing cyanobacteria generally have a bigger gene family encoding the FDPs. Six genes could be found in genome of *Anabaena* sp. PCC 7120. Four of them, *flv*1b, *flv*2, *flv*3b and *flv*4 are highly similar to corresponding genes in *Synechocystis,* but the two extra genes, *flv*1a and *flv*3a, are slightly more different from corresponding *flv1* and *flv*3 in *Synechocystis*. We attempted to determine the reasons for duplication of the *flv*1 and *flv*3 genes in *Anabaena*.

The results of RT-qPCR show clear differences in the expression behaviour of the *flv*1b and *flv*3b genes compared to *flv*1a and *flv*3a, respectively, in all experiments performed so far. The expression level of the '*b*' genes increased upon a shift of cells from high to ambient CO_2 level and also during the high light treatment independently of the source of nitrogen. On the contrary, the transcription levels of the '*a*' genes depended only on the availability of combined nitrogen in the growth medium, with strong induction occurring upon nitrogen deprivation. Fusion of target proteins with YFP confirmed that Flv1A and Flv3A are localized exclusively in heterocysts, while Flv1B and Flv3B are present in vegetative cells.

We have constructed mutants lacking the *flv*1a, *flv*1b, *flv*3a, and *flv*3b genes. Δ*flv*1a and Δ*flv*3a mutants demonstrated strong phenotype at N_2-fixing conditions. These mutants demonstrated light-induced oxygen uptake whereas it was completely inhibited in Δ*flv*1b and Δ*flv*3b.

We suggest that the flavodiiron proteins Flv1B and Flv3B perform photoreduction of O_2 in vegetative cells of *Anabaena*, but Flv1A and Flv3A are related to N_2 fixation.

P03-33
Operon *flv4-flv2* provides cyanobacteria with a novel photoprotection mechanism

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The genome of *Synechocystis* sp. PCC 6803 comprises four FDPs (flavodiiron protein) genes: *flv1 (sll1521), flv3 (sll0550)* and*flv2* (*sll0219*) and *flv4* (*sll0217*), with the last pair organized in the operon together with the *sll0218* gene.

Expression of the *flv2* and *flv4* genes is strongly induced under air level of CO(LC condition) at both the transcript and protein levels. The most rapid induction occurs at LC conditions and high light irradiance (1). In the Δ*flv2* and Δ*flv4* mutants grown in LC conditions, a strong light-dependent decline in functional PSII centers was observed, suggesting that Flv2 and Flv4 are crucial for protection of PSII centers against photoinhibition (1).

The Flv2 and Flv4 proteins form a heterodimer which is localized in cytoplasm but also has a high affinity to membrane in the presence of divalent cations. Sll0218 resides in the thylakoid membrane in association with yet unidentified high molecular mass protein complex. Biophysical measurements performed with different deletion mutants provided evidence of a novel electron transfer pathway to the Flv2/Flv4 heterodimer from PSII and an important role of Sll0218 in the stabilization of the PSII dimers (2).

To further investigate this hypothesis, we overexpressed in *Synechocystis* the native *flv4-sll0218-flv2* operon. In order to reveal possible interacting partners, other overexpression constructs with various affinity tags at the N- and C-termini of genes of the *flv4-2* operon were obtained. Biochemical and biophysical analyses performed with these mutants highlight the important role of Flv4, Sll0218 and Flv2 in photoprotection of PSII.

The *flv4-flv2* operon provides many cyanobacteria with a new type of photoprotective mechanism, which is evolved in parallel with oxygen evolving PSII.

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P03-34
CRK5 as a convergence node between senescence and abiotic stress responses

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In plants, receptor-like protein kinases (RLKs) play essential roles in signal transduction by recognizing extracellular stimuli and activating the downstream signalling pathways. Cysteine-rich receptor-like kinases (CRKs) constitute a subfamily of RLKs and are distinguished by the novel C-X8-C-X2-C motif (DUF26) in the extracellular domains. In *Arabidopsis thaliana*, 44 members of CRKs family have been identified. One of them, CRK5, raises particular interest as a putative negative regulator of senescence and stress acclimation response. Functional characterization of *crk5* shows its impaired adaptation to abiotic stress – UVC radiation, as well as decreased chlorophyll content, lower biomass production and visible symptoms of premature leaf aging. Accelerated senescence of *crk5* is even more induced by external stimuli such as continuous dark and low CO_2 concentration.

CRK5 gene has many W-Box cis-elements in its promoter region and therefore it may act as a downstream target gene of some WRKY transcription factors e.g. WRKY53 and 70 which are well known regulators of both plant senescence and defense response.

P03-35
Redox control in the adaptation of plants to elevated light intensities

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Plants adapt to their light environment through regulation of enzymatic activities and modulation of gene expression in order to optimise photosynthetic productivity under low light conditions and to avoid damage caused by reactive oxygen species under high light conditions. The acclimation responses involve e.g. changes in the photosystem II (PS II) antenna size through long-term adjusments of the amounts of light-harvesting complex II (LHC II) proteins per PS II unit. A sudden increase in light intensity elicits a response termed non-photochemical quenching (NPQ) of chlorophyll fluorescence. This implies thermal dissipation of part of the light energy absorbed by LHC II, thus avoiding overexcitation of PS II and the concomitant production of reactive oxygen species. Given that the redox states of thioredoxins (Trx) depend on the light conditions, these enzymes have been suggested as possible transmitters in the signalling leading to light acclimation. We have studied the responses to elevated light intensities of *Arabidopsis thaliana* knockout mutants lacking the NADPH thioredoxin reductase C (NTRC), 2-Cys peroxiredoxins or Trx *x*. The capacity to adapt to various light conditions was assessed by analyses of growth, chlorophyll fluorescence, contents of chlorophylls and carotenoids and measurements of the proportions of LHC II and PS II proteins. The results suggest that the NTRC enzyme is involved in the redox regulation of the NPQ thermal dissipation of light energy, since the NTRC knockout mutant displays extremely high levels of NPQ even under moderate light intensity and, in agreement with these data, shows altered levels of the xanthophyll pigments, violaxanthin, antheraxanthin and zeaxanthin.

P03-36
Purification and characterization of a lectin from the hand flower tree *(Chiranthodendron pentadactylon)*

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Chiranthodendron pentadactylon has been used in traditional medicine to treat wide variety of ailments, generally is used in the form of infusion (tea) The pharmaceutical analyses indicate that the infusion have antibacterial, antiviral and antiparasitic properties, also exhibit anticolinergic and vasorelaxant activity attributed to flavonoids. The presence of lectins in the crude extract was verified using human blood types. The highest activity was obtained when using human blood type A_2 (N-Acetylgalactosamine-Galactose-Fucose). The pure lectin was named '*Chiranthodendron pentadactylon* lectin CpL' which exhibit the following characteristics: apparent molecular weight: 32 812 kDa; Isoelectric point PI: 5.01; The lectin (CpL) secondary structure exhibit alpha helix, analyzing the ellipticity obtained between 210 and 218 nm when using circular dichroism. This lectin is 19% glycosilated. The specificity for monosaccharides is the following: Man > Gal > GalNAc > GalAcet > NANA > Lac > Glc, GlcNAc > Fruc > Fuc > Rib > Sac > Xyl. With cation dependency to Ca^{++}, K^{+} y Na^{+}. And a stability in the range of pH 5.8–7.6 and 17–60°C. The crude extract exhibit antibacterial activity against *Escherichia coli* ATCC 25922 (MIC: 214 μg/ml) and against *Staphylococcus aureus* ATCC 25923 (CIM: 7 μg/ml), the pigments presented antibacterial activity against *E coli* and the protein fraction was active against *Pseudomonas aeruginosa*. ATCC 27853. This is the first study where the purification and, characterization of a lectin (CpL) from *Chirandodendron pentadactylon* is reported.

P03-37
Changes in volatiles during grape maturation of two autochthon Castilla-La Mancha varieties: Airen and Tempranillo

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The secondary metabolites of grapes (*Vitis vinifera*) play a key role in wine quality. The phenolic components of the skin and seeds are the main source of wine colour and its structural properties, while the volatile components are the major determinants of aroma and flavour in wine.

Wine aroma is formed by both natural volatile substances synthesized during fermentation and complex aromatic substances derived from maturation during aging processes. The final aroma of wine is determined by several hundred volatile compounds of varying chemical nature.

The aim of this study was to characterize the evolution of the aromatic compounds during the berries maturation of two autochthon varieties of grape from Castilla-La Mancha: Airen and Tempranillo. Samples were collected at different developmental stages from Tarazona de la Mancha, Spain during 2010 and 2011 from July to October. To analyze the volatile compounds in fruits, 1 g fresh weight was incubated at 30°C for 10 min and then the samples were analyzed by HS-SPME-GC-MS. Each compound was unequivocally identified by comparison of both mass spectrum and retention time. The principal component analysis (PCA) was carried out with the aim of highlighting the main contributors to the variance among samples.

The results showed that both varieties have specific families of volatiles, which are characteristic of each specific stage. For example, the terpenoide family has been identified only in immature stages, while the family C6 compounds have been detected in harvest mature stage. The relationship between grapes maturation and volatiles contents is discussed.

P03-38
Identification of a 2-cys peroxiredoxin as a tetramethyl benzidine-hydrogen peroxide stained protein from the thylakoids of the extreme halophyte *Arthrocnemum macrostachyum* L.

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Tetramethylbenzidine-H_2O_2 staining of SDS-polyacrylamide gel is a widely used method for the specific detection of proteins with heme-dependent peroxidase activity. When this method was used with thylakoids from the halophytic plant Arthrocnemum macrostachyum, besides the cytochrome f and cytochrome b6 proteins usually found in higher plants and cyanobacteria, at least four additional bands were detected. One of them, a 46-kDa protein, was shown to be an extrinsic protein, and identified by mass spectrometry and immunoblotting as a 2-cys peroxiredoxin. Peroxidase activity was insensitive to oxidizing agents such as trans-4,4-diydroxy-1,2-dithiane or hydrogen peroxide, but was inhibited by treatment of thylakoids with reducing agents such as dithiothreitol or mercaptoethanol. By immunoblotting, it was shown that loss of peroxidase activity was paralleled by disappearance of the 46-kDa band, which was converted to a 23-kDa immunoreactive form. A dimer/monomer relationship between the two proteins is suggested, with the dimeric form likely being a heme-binding protein. This possibility was further supported by anionic exchange chromatography and de novo sequencing of tryptic fragments of the protein and sequence comparison, as most of the residues previously implicated in heme binding in 2-cys peroxiredoxin from Rattus norvegicus were conserved in *Arthrocnemum macrostachyum*. The amount of this protein was modulated by environmental conditions, and increased when salt concentration in the growth medium was higher or lower than the optimal one.

P03-39
Antioxidant activities and total phenolic compound levels of various plants

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Antioxidant activities and polyphenol contents of 15 plants (Achilla millefolium-Acm-, Agropyron repens-Agr-, Crataegus monogyna fruit-Crmf, Crataegus monogyna leaves-Crml-, Humulus lupulus-Hul-, Juniperus communis-Juc-, Lavandula stoechas-Las-, Lepidum sativum- Les-, Linum usitatissimum-Liu-, Nigella sativa-Nis-, Papaver rhoeas-Par-, Plantago major-Plm-, Trigonella foenum-Trf-, Urtica dioica-Urd-, Viscum album-Via-) were investigated.

Initially, plants were exposed to extraction in water, 80% ethanol and 80% methanol. 'DPPH radical scavenging assay' for antioxidant activity (AA) and 'Folin-Ciocalteu reagent assay' for total phenolic compound (TPC) levels were carried out in the extracts. The values of AA and TPC were found to be higher with methanol extraction, mostly. AA values (% inhibition) of the six of the plants (Acm, Crml, Las, Par, Plm, Urd) were found to be >85%. The plants with the highest TPC content were Crml, Las and Plm (32.93; 25.00 and 22.36 GAE/g, respectively).

By comparison of HPLC chromatograms of the raw extracts with HPLC chromatograms of the standards chosen for the study (Biochanin A-BA-, Caffeic acid-CAF-, Catechin-CAT-, Chlorogenic acid-CHA-, Daidzein-DZ-, Ferulic acid-FER-, Formononetin-FMN-, Gallic acid- GA-, Genistein-GEN-, 6-Methoxyflavanon-MF-, o-Coumaric acid-oCA-, p-Coumaric acid-pCA-, Protocatechuic acid-PA-, Quercetin-QU), 8 of the standards were determined in various amounts in 8 of the plants (CAF in Acm, Plm, and Crml; CHA in Acm, Crml and Urd; FER in Trf; GA in Plm, Crml and Urd; MF in Las; oCA in Les, Acm and Trf; PA in Urd; QU in Les, Las, Crml and Hul).

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Keywords: antioxidant activity, extraction, polyphenols, Achilla m., Agropyron r., Crataegus m., Humulus l., Juniperus c., Lavandula s., Lepidum s., Linum u., Nigella s., Papaver r., Plantago m., Trigonella f., Urtica d., Viscum a., methoxyflavanon, o- quercetin, caffeic, chlorogenic, coumaric, ferulic, gallic, protocatechuic acid.

P03r-40
Redox control of the carbon flux in cereal endosperm

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Cereal seeds store large amounts of starch, which serves as energetic reserve for initial stages of seedling growth upon germination. Starch synthesis in endosperm of cereal grains has been intensely studied due to its great economic importance. Starch biosynthesis in heterotrophic tissues depends on sucrose imported from photosynthetic tissues, which is transformed by different enzymes into both glucose-6-phosphate and ADP-glucose in the cytosol, and then imported into the amyloplast for starch synthesis. Despite the sound knowledge of the enzymes involved in this pathway, there are many open questions concerning the signals and mechanisms regulating starch metabolism. This work is focused on the redox regulation of the enzymes participating in the carbon flux in cereal endosperm, particularly on the role of NTRC in the redox control of starch metabolism. NTRC, a plastidial NADPH-thioredoxin reductase (NTR) with a thioredoxin

domain, is involved in the redox control of AGPase, a key regulatory enzyme of starch synthesis both in leaves and roots in Arabidopsis (1). Here, we show that NTRC is also present in endosperm of cereal grains. Moreover, we have performed a search of NTRC targets in this tissue based on affinity chromatography with a monocysteinic NTRC mutant and immunoprecipitation followed by identification by mass spectrophotometry. Results demonstrate that NTRC interacts with proteins participating in starch biosynthesis, thus, suggesting an important function of NTRC in the redox regulation of this pathway.

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P03-41
Plasticity of the response of repetitive/non coding DNA to the heavy-metal stress

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Previous works have shown that genome reacts to heavy-metal stress by selective amplification of (G + C)-rich highly repetitive/non coding DNA. Such phenomenon takes place similarly both in protonemal and in leafy shoot phases. However, when metals are removed from culture medium, if stress has taken place during the leafy shoot phase new-formed DNA is eliminated from nucleus; and vice versa, if stress has taken place during protonemal phase, the new-formed DNA remains inside genome of the target organism.Our idea is that such different behaviour of metal-induced repetitive/non coding DNA could be linked to different role of these two phases: in fact, while in protonemal phase protective role of repetitive/non-coding DNA may have a particular importance, conversely, in leafy shoot phase preservation of new generation from genome's changes may have the priority. Based on these considerations, we have analyzed the behavior of metal-induced repetitive DNA during transition from protonemal to leafy shoot phase in different kind of situations. Our results have showed that: (i) most of repetitive/non coding DNA sequences triggered by heavy-metal stress in protonemal phase, even if apparently it should be stabilized in the genome, it is not actually transmitted to leafy shoots phase; (ii) in the leafy shoots resulting from stressed protonemata, answer of repetitive DNA to metal stress is similar but much less meaningful compared to the one trigged by the same conditions in the leafy shoots resulting from unstressed protonemata; (iii) in the leafy shoots resulting directly from those from mine through vegetative way, heavy-metal stress doesn't trigger any type of DNA amplification. Some hypotheses are discussed on the basis of observed morphological and functional modifications.

P03-42
Elucidating the role of NTRC in chloroplast redox homeostasis based in the analysis of proteins interacting *in vivo* with NTRC in *Arabidopsis*

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Redox status in chloroplasts is maintained through the activity of a set of thioredoxins, with reducing power from Fd, reduced by the electron transport chain, and catalysed by FTR (ferredoxin-dependent thioredoxin reductase). In addition, NTRC (NADPH thioredoxin reductase C), a peculiar NTR with a thioredoxin domain at the C-terminus, recently identified as an enzyme capable of efficiently reducing 2-Cys-Prx using NADPH as source of reducing power, plays a key role in redox regulation in this organelle. The fact that the deficiency of NTRC in Arabidopsis *ntrc* mutant plants causes a much more severe phenotype than that of the double knock down mutant of 2-Cys-Prx (1), points out that NTRC must have additional functions. Some of these, including the regulation of starch (2), aromatic amino acid and auxin synthesis (3), have already been identified.

With the aim of establishing the function of NTRC in chloroplast redox homeostasis, we have developed a strategy based in TAP tagging, in order to identify proteins interacting with NTRC in the intracellular redox conditions. A tagfused to NTRC at the C-terminus, allows the purification of NTRC-containing complexes through two steps of affinity purification separated by digestion with 3C protease (4). We have generated transgenic plants over-expressing NTRC fused to the tag and established a protocol for purification of chloroplast-localized NTRC complexes. By this strategy, we have identified proteins involved in different metabolic pathways such as carbohydrate and energy metabolism, protein turnover and post-translational modification.

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P03-43
Integrating redox signals in photosynthetic and non-photosynthetic tissues in *Arabidopsis thaliana*

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Redox regulation based on dithiol-disulphide interchange is an essential component of the control of chloroplast metabolism in which NADPH-dependent thioredoxin reductase C (NTRC), a chloroplast-localized NTR with a joint thioredoxin domain, plays an important role (1). Recently we have found that NTRC is also located in plastids of non-photosynthetic tissues (2), which lack photochemical reactions, so that their redox homeostasis depends exclusively on NADPH produced from sugars. In this study, we have tested the possibility that the functions of chloroplasts and non-green plastids, such as amyloplasts, are integrated to harmonize the growth of the different organs of the plant. To that end, we generated Arabidopsis plants with recovered redox homeostasis exclusively in chloroplasts or in amyloplasts, by leaf- or root-specific expression of NTRC in the ntrc mutant, respectively. The analysis of these plants suggests that chloroplasts exert a pivotal role on plant growth, as expected because chloroplasts constitute the major source of nutrients and energy, derived from photosynthesis, for growth of heterotrophic tissues. However, NTRC deficiency causes impairment of auxin synthesis and lateral root formation. Interestingly, recovery of redox homeostasis of chloroplasts, but not of amyloplasts, was sufficient to restore wild type levels of lateral roots, showing the important signalling function of chloroplasts for non-green organ development.

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P03-44
Molecular mechanism underlying nitrate-responsive gene expression in *Arabidopsis*

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Nitrate is a major nitrogen source for land plants and also acts as a signaling molecule that induces various changes in growth and gene expression. For instance, a nitrate supply immediately induces the expression of genes encoding nitrate reductase (NR) and nitrite reductase (NIR), which catalyze reactions for nitrate reduction in the nitrate assimilation process. Despite the fact that nitrate is a critical nutrient signal regulating gene expression in plants, the cis-regulatory elements and trans-acting factors for nitrate-responsive gene expression in higher plants have not been identified yet. Here we report that identification of nitrate-responsive cis-element (NRE) and proteins interacting with NRE. By analysis using GUS reporter gene and comparison of the sequences of several NIR gene promoters from various higher plants, a 43-bp sequence in the promoter of Arabidopsis nitrite reductase gene (NIR1) was found to be necessary for nitrate-inducible gene expression. Indeed, a synthetic promoter in which the four copies of a 43-bp sequence were fused to the 35S minimal promoter did direct nitrate-responsive transcription, indicating that the 43-bp sequence is an authentic NRE. On the other hand, we also found that the nitrate-inducible expression of an Arabidopsis NR gene, *NIA1*, is regulated by the 3' flanking sequence of the gene, which included two sequences similar to the NRE found in the NIR1 gene promoter. We recently identified proteins interacting with the NRE in the NIR1 gene promoter and found that these proteins could bind the putative NRE sequences in the 3' flanking sequence of *NIA1* as well. Based on these findings, we will discuss roles of NREs and NRE-interacting proteins in nitrate regulation in *Arabidopsis*.

P03-45
Application of rhodamine and betaine – based fluorescent dyes for studying protein expression in *Malus domestica* with 2D gel electrophoresis

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Proteomics can provide valuable evidence for the elucidation of mechanisms of important cellular processes of plants. Gel electrophoresis offers good resolution of complex protein samples and has been applied to the study of various processes, like seed development in soybean (3) or salicylic acid response in cucumber (4).

Saturating labeling with maleimide-conjugated dyes, which covalently label the cysteine residues of proteins, offer high sensitivity and are applicable for small protein samples, for example those obtained from leaf disks or by subcellular fractionation. These dyes are expected to give good coverage of *M. domestica* proteins, since 93% of the predicted proteins from the *M. domestica* genome sequence (2) contain a cysteine amino acid.

New rhodamide and betaine – based fluorescent dyes have recently been applied for the study of human keratinocytes (1). These dyes offer a cost-effective alternative to the widely used cyanine dyes. We compared the electrophoretic mobility and isoelectric points of Dy-555, Dy-560 and Dy-635 – labelled proteins. The isoelectric points of Dy-560 and Dy-635 – labelled proteins were found to be very similar, but the electrophoretic mobility differed slightly, especially in the low molecular weight region.

Phenol extraction of proteins from single 0.5 cm^2 leaf disk provided protein yields (62 ± 7 μg for cv. Gala, 59 ± 7 μg cv. Orlovim) sufficient for labeling reaction and sensitive detection with gel electrophoresis.

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P03-46
Molecular regulation of chlorophyll a fluorescence decay and foliar heat dynamics in *Arabidopsis*

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In a simplified model of photosynthesis, light energy absorbed by chlorophylls of photosystem II is distributed between photochemistry, fluorescence and heat. Time-resolved fluorescence combined with foliar heat dynamics measurements demonstrates that higher plants evolved genetic and physiological regulatory system, which optimizes photosystem II quantum-molecular functions and the fate of absorbed photons in excess. In leaves exposed to excess light (EL) chlorophyll *a* fluorescence decay time (FDT) was reduced and after 60 min of recovery in low light (LL) it reached its initial level. During repetitive EL/LL cycles in thermal experiment, it heated and cooled in a very stable manner. Dynamics of these processes was differentially deregulated in recessive null mutants (*npq4-1*, *cao* and *ffc1-2*) for genes encoding PsbS, an important protein for non-photochemical quenching, and the chloroplast signal recognition particle system (cpSRP54 and cpSRP43), respectively. Chaotic changes of FDT in *npq4-1* and *cao* during the LL recovery was associated with unstable foliar heat dynamics. Shortened FDT after EL incident in *ffc1-2* extended very slowly and it did not reach the initial level even after 2 hour of recovery in LL. It also had more stable foliar heat dynamics than the wild type during repetitive EL/LL cycles. Our results indicate the role of the PsbS and photosystem II antenna organization in the regulation of FDT and foliar heat dynamics. This work was supported by the Welcome/2008/1 and Welcome/2008/2 Programs operated within the framework of the Foundation for Polish Science, co-financed by the European Regional Development Fund given to S.K and S.M, respectively.

P03-47
Fluorescence studies of *Arabidopsis thaliana* exposed to excess red radiation

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Energy absorbed by plants is divided into photochemistry, heat dissipation, and fluorescence. Any changes in this distribution induced by excess light influence intensity and decay of PS II fluorescence. Here we study the effect of excess light upon the fluorescence of Arabidopsis and three recessive null mutants (*npq4-1*, *cao* and *ffc1-2*) in order to demonstrate genetic and molecular nature of regulation of the fate of absorbed photons. Illuminating halves of rosettes with strong 620 nm light causes minute changes of the fluorescence decay in native plants. In contrast in the case of *npq4-1* mutant it leads to strong reduction of the decay. Importantly, the second half of the rosette that was not exposed to the excess light feature lifetime located between low-light adapted and excess light treated leaves. Similar qualitative effects were observed also for the remaining two mutants, albeit the differences were smaller. The result indicates that excess light results in huge changes of excitation energy dissipation in plants. Analysis of behaviour measured for the whole set of genotypes indicates the role of the PsbS and photosystem II antenna organization in efficient and discrete global regulation of the rates between photochemistry, fluorescence, and heat.

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P03-48
Purification, partial characterization and *in vitro* biological activity against pest insects of a novel trypsin inhibitor from *Sapindus saponaria* seeds

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Protease inhibitors have important roles in plant defense and show biological properties of interest for biotechnological applications in agricultural and medical areas. The aim of this work was to purify and characterize a new trypsin inhibitor from *Sapindus saponaria* seeds, and evaluate its *in vitro* biological effect on the digestive enzymes of pest and disease vector insects. The new trypsin inhibitor from *S. saponaria* seeds detected in this study was named SSTI2, and was purified by protein precipitation with trichloroactetic acid, affinity chromatography and reverse phase chromatography using UFLC system. It is a member of Potato I class of serine protease inhibitors, which is recognized by their toxicity upon insects. Molecular mass (7.571.976 Da) and primary structure (under patent registration) of SSTI2 were determined by means of mass spectrometry (MALDI-TOF and ESI-TOF). SSTI2 is a strong trypsin inhibitor ($IC_{50} = 8.3 \times 10^{-2}$ μM), but it was not able to significantly inhibit chymotrypsin and papain (13 and 5%, respectively) and shows no bromelain inhibitory activity. SSTI2 showed high inhibitory effect on trypsin from the midgut of the disease vector insect *Aedes aegypti* and the pest insects *Anthonomus grandis* and *Spodoptera frugiperda* (about 92, 78 and 71%, respectively), and lower inhibition of trypsin from the midgut of *Anticarsia gemmatalis* (about 32%). This significant *in vitro* inhibitory effect of digestive enzymes suggests that suppression of the development and decrease in survival of these insects fed with diets containing SSTI2 may occur. Further investigations are needed to best characterize SSTI2 and determine its potential application as a new biotechnological tool, especially as a new insecticide against *A. aegypti*, *A. grandis* and *S. frugiperda*.

P03-49
A rubisco mutant of *Chlamydomonas reinhardtii* that enhances photosynthetic hydrogen production via photosynthesis

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Molecular hydrogen (H_2) has been increasingly regarded as an interestingalternative source of energy owing to a higher specific enthalpy of combustion and absence of polluting gases as products. This form of biofuel can be released by microalgae via reduction of free protons to molecular hydrogen catalyzed by hydrogenases. The main competitor for the reducing power required by hydrogenases is the Calvin cycle, and the enzyme rubisco plays a key role therein. A mutant strain of *Chlamydomonas* with reduced rubisco level, activity and stability was used in this research effort aimed at increasing the H_2 productivity. Biochemical characterization of such metabolically engineered mutant cells proceeded in TAP culture medium, with sulfur-depletion or sulfur-repletion – both under hypoxia conditions; the chlorophyll, protein and starch contents were all measured. In addition, the expression of rubisco, Fe-hydrogenase, D1 and Lhcb were investigated, besides quantifying H_2. Differences in the patterns of expression of those proteins were observed between the mutant and control strain. Rubisco in mutant cells was present to very low levels and underwent intense degradation, and Lhcb proteins exhibited monomeric isoforms during the first 24–48 hour, whereas D1 displayed a notorious sensitivity especially under sulfur-depletion. Higher levels of Fe-hydrogenases were attained in this rubisco mutant when compared with the control. Although the sulfurdepleted medium was much more suitable than its whole counterpart for H_2 production, sustained photosynthetic hydrogen release was observed in sealed cultures of rubisco mutant cells in complete TAP medium, under moderate light conditions. Our experimental results indicate that rubisco is a promising target to improve hydrogen production rates in engineered photosynthetic microorganisms.

P03-50
Bitter fennel essential oils from Spain with antioxidant and inhibitory activity on tyrosinase

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Main biomolecules of bitter fennel (BF) or *Foeniculum vulgare* were identified by GC-MS. Most abundant compounds of BF from Murcia were α-Phellandrene (25.64%), Myrcene (19.36%) and α-pinene (10.13%), whereas main components of BF from Huelva were Limonene (28.49%), trans-Anethole (24.39%) and Fenchone (17.76%). So we considered that BF from Murcia is Phellandrene type in contrast to the plant from Huelva which is Anethole type, according to the ISO standards.

Then, global antioxidant activity of BF essential oils was determined by the ORAC and β-carotene bleaching assays, using Trolox Equivalent Antioxidant Capacity (TEAC) units. Using the ORAC method, antioxidant activity values of these plants were 0.304 TEAC in BF from Murcia, and 0.675 TEAC in BF from Huelva. These results were different from results obtained by β-carotene bleaching assay, which showed a higher antioxidant capacity of BF from Murcia (0.261 TEAC), and lower activity in the plant from Huelva (0.101 TEAC). Thus, we could check that these kinds of essential oils contain distinct biomolecules, which show different activity as scavengers of peroxyl, linoleate and β-carotene free radicals.

Finally, we proved that BF essential oils have a global competitive type inhibitory ability of diphenolase activity of mushroom tyrosinase enzyme, according to the Dixon Graphic. The apparent inhibition constants values were 242 ppm in BF from Murcia, and 293 ppm in case of the plant from Huelva. In conclusion, the enzyme has the highest affinity towards the essential oil of BF from Murcia.

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P03-51
Purification and characterisation of β-glucosidase from olive *(Olea europaea* L.*)*

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β-glucosidase is a glucosidase enzyme that acts upon β1- > 4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose). It is an exocellulase with specificity for a variety of beta-D-glycoside substrates. It catalyzes the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose. An olive *(Olea europaea* L.*)* β-glucosidase was purified to apparent homogeneity by salting out with ammonium sulfate and using specifically designed sepharose-4B-L-tyrosine-1-napthylamine hydrophobic interaction chromatography. The purification was 197.23 fold with an overall enzyme yield of 76.61%. The molecular mass of the protein was estimated as 65 kDa. The purified β-glucosidase was effectively active on p-/o-nitrophenyl-β-D-glucopyranosides (p-/o-NPG) with K(m) values of 5.6 mM and *V*(max) values of 666 6667 U/mg. The enzyme was competitively inhibited by NaOH and sitric acid against p-NPG as substrate. The IC50 values of NaOH were determined as 68 160 mM while the enzyme was more tolerant to sitric acid inhibition with IC50 values of 61 073 mM respectively, for p-NPG.

Keywords: Olive, β-glucosidase, hydrophobic interaction chromatography

P03-52
The strawberry *FaGAST2* gene determines receptacle cell size during fruit development and ripening

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Numerous GAST-like genes have been reported in higher plants but only one *GAST*-like gene (*FaGAST1*) has been described in strawberry so far. In this paper, we have identified a novel strawberry *FaGAST* gene (*FaGAST2*) whose expression showed an increase throughout fruit receptacle development and ripening, coinciding with those stages where a decrease in fruit expansion processes (G3-W and R-OR stages) occurs. *FaGAST2* only shares 31% and 15.7% of their amino acid and nucleotide sequence homology respectively with the previously reported *FaGAST1* gene but both genes contain a signal peptide and a highly conserved GASA domain (cysteine-rich domain) in the C-terminal region. *FaGAST2* expression is mainly confined to the fruit receptacle and is not regulated by auxins, GA_3 or ABA but is regulated by etephon, an intracellular generator of ethylene. In addition, the expression of *FaGAST2* gene also increased under oxidative stress conditions (H_2O_2 or *Colletotrichum acutatum* infection), suggesting a direct role of FaGAST2 protein in ROS scavenging during fruit growth and ripening and during the fungal infection. On the other hand, the over-expression of *FaGAST2* gene in different transgenic lines analyzed caused a delay in the growth of strawberry plants and a reduction in the transgenic fruits size. The histological studies performed in these fruits showed that their parenchymal cells were smaller than their control ones supporting a relationship between the *FaGAST2* gene expression, the strawberry fruit cell elongation and the fruit size.

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P03-53
α-Hairpinins, a novel family of plant defense peptides

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Plant defense peptides are a heterogenic group of peptide compounds that play an important role in plant resistance to stress. Most defense peptides are believed to directly inactivate pathogens or deter pests. Probably the best studied groups of defense peptides in plants are antimicrobial peptides (effective against pathogens) and protease inhibitors (active against insect pests). Here we report on our investigation of a new family of plant defense peptides termed α-hairpinins. The primary structure of

these peptides contains a characteristic cysteine motif $C^1X_3C^2X_nC^3X_3C^4$, where X is any residue; the four strictly conserved cysteine residues form two intramolecular disulfide bridges C^1–$C^4$and C^2–C^3. α-Hairpinins have been found in all flowering plants that we investigated so far and seem ubiquitous; at present we have solid data on sequence and biological activity of 15 peptides from six different plant families. The best studied are the trypsin inhibitor BWI-2c (41 residues) from buckwheat (*Fagopyrum esculentum*) and antimicrobial peptide EcAMP1 (37 residues) from barnyard grass (*Echinochloa crus-galli*). Successful production of recombinant BWI-2c and EcAMP1 in *Escherichia coli* allowed us to conduct thorough structural and functional investigations. BWI-2c was shown to effectively inhibit trypsin-like enzymes from insect digestive systems ($K_i \sim 10^{-10}$ M), whereas EcAMP1 exhibited high antifungal activity against phytopathogenic fungi ($EC_{50} \sim 1$–20 μM). The three-dimensional structure of both peptides was determined by NMR. They share a common fold represented by an α-helical hairpin (hence the name of the family) with two antiparallel α-helices stapled by two S-S bridges. The structure of α-hairpinin genes suggests their complex evolution. For instance, in some cereals α-hairpinins are coded in tandem repeats, whereas in many different plants they are produced as parts of larger precursors for storage proteins. The new family of plant defense peptides is an interesting object of structure-function investigations and a tool of resistance engineering in crops.

P03-54
Insight into the molecular mechanism of binding to antibiotics and antimicrobials of the multidrug binding protein TtgR

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The biotechnological value of the Pseudomonas Putida species derives from its high resistance to toxic compounds. One of the major mechanisms that underline this resistance is the active extrusion of toxic compounds through membrane-bound efflux pumps, which are regulated at the transcriptional level. TtgR is a HTH-type transcriptional regulator of TtgABC efflux pump of Pseudomonas putida DOT-T1E which presents metabolic capabilities and exhibits resistance versus many different antibiotics, solvents and flavonoids. Structurally, TtgR is a dimeric protein with a big cavity al the active site that confers its multidrug binding potential and therefore it is responsible for the resistant phenotype of Pseudomonas putida DOT-T1E. Previous studies have shown its high binding affinity for plant-derived compounds as phloretin and moderate affinity for other effectors such as chloramphenicol. The binding of a single effector molecule induces the dissociation of the repressor-operator complex. One of the main strategies to modulate the bacterial resistance to antibiotics, antimicrobial and toxic compounds is the rational modification of the target binding site. For this reason, it is of great interest to gain insights on the molecular mechanism that controls the binding of this protein to different compounds. Since, the presence of aromatic rings and hydroxyl groups in the ligands is crucial for binding to TtgR, we have designed four mutants al the active site: S77A, E78A, N110A, and H114A. A comprehensive knowledge of the key features in the efflux system of the bacteria would be highly beneficial in advanced drug design and improvement of the plant-microorganism-environment interaction. We will present the biophysical characterization of these mutants.

P03-55
H1.3 linker histone in adaptation to stress in *Arabidopsis thaliana*

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In nuclei of eukaryotic cells genomic DNA is packed into chromatin by histones and non-histones proteins. The basic unit of this structure is a nucleosome, which consists of ca. 200 base pairs of DNA associated with core histones (H2B, H2A, H3, H4) and linker histone H1. In contrast to core histones, the biological function of linker histone variants in chromatin is not well understood. It has been suggested that in both plants and animals they may control gene expression by affecting DNA methylation and the formation of higher-order chromatin structure (1–3). In Arabidopsis there are three non-allelic variants of H1, two major variants H1.1, H1.2 and a stress-inducible variant H1.3. Knockout of any single H1 variant seems to have no effect on viability of plants under normal conditions. Our analysis of 2000 base pair of H1.3 promoter showed that it includes sequence motifs responsible for light regulated transcription, abiotic and biotic stress-induced transcription as well as other motifs. We present results using h1.3 mutant and H1.3-GFP lines that verify hypotheses on H1.3 involvement in Arabidopsis adaptation to stress.

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P03-56
Analysis of expression and identification of interacting partners of *ATSWP73A* and *ATSWP73B* subunits of *Arabidopsis* SWI/SNF-type chromatin remodeling complex

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The ATP-dependent SWI/SNF chromatin remodeling complexes change the structure of chromatin and modulate DNA accessibility during transcription, replication and DNA repair.

Despite intensive studies, the current knowledge about plant SWI/SNF complexes is still limited.

In *Arabidopsis* genome occur four genes encoding SWI2/SNF2 ATPases, four *SWI3* genes – *ATSWI3A, ATSWI3B, ATSWI3C, ATSWI3D* (instead of two in yeast and mammals), a single copy of *SNF5* (named *BSH*) and two copies of *ATSWP73* genes. So far, there is no data concerning the activity and role of ATSWP73A or ATSWP73B in *Arabidopsis*.

We are involved in genetic and biochemical characterization of *Arabidopsis* SWI/SNF complexes carrying ATSWP73A or ATSWP73B subunits. We showed using Yeast-Two-Hybrid assay that ATSWP73A interacts with ATSWI3C. We also have data indicating that ATSWP73 proteins are involved in various regulatory processes during plant growth and development.

There are no homozygous mutant lines with T-DNA insertion in the *ATSWP73A* gene, available in SALK or other collections. We therefore construct *Arabidopsis* lines carrying inducible SWP73amiRNA constructs capable of silencing each or both of the *ATSWP73* genes. The expression analysis using *GUS* gene showed that *ATSWP73* are ubiquitously expressed in almost all tissues of plants throughout their whole life.

Our findings reveal the so far unrecognized biological function of ATSWP73 proteins in plants.

P03-57
ATSWI3C subunit of the Arabidopsis SWI/SNF chromatin remodeling complex is involved in the GA response pathway

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SWI/SNF-type chromatin-remodeling complexes (CRCs) play pivotal roles in the regulation of basic cellular processes, including transcription, DNA replication, repair and cell cycle in eukaryotes. Although several orthologues of CRC components are conserved between higher plants and other eukaryotes, our current knowledge on plant CRCs is rather limited.

Here we report that ATSWI3C, the core component of Arabidopsis SWI/SNF CRCs is directly involved in the GA signalling pathway. We found that the ATSWI3C subunit of Arabidopsis SNF/SWI CRC interacts *in vivo* with ATSWI3B and two DELLA repressors RGL2 and RGL3.Some phenotypic traits of the *atswi3c* null mutant, including the seed coat structure, resemble those of GA deficient mutants.The *atswi3c* mutant shows hypersensitivityto GA treatment, and acceleration of flowering and seedlings growth. Transcription of several genes in the GA biosynthesis and signaling pathways is dependent on ATSWI3C-containing SWI/SNF CRCs. Moreover, the effect of *atswi3c* mutation on expression of cell cycle-related genes is similar to that of the *ga1-3* mutation. Taken together, our data indicate that, similarly to BRM and ATSWI3B, ATSWI3C is involved in the regulation of GA responses and probably acts together with ATSWI3B and BRM as controlling the expression of some DELLA target genes.

A.R., E.B. and E.S. contributed equally to this work; T.J.S. and A.J. corresponding author, contributed equally to this work.

P03-58
Strategy for cloning of oxidosqualene cyclases from Maytenus ilicifolia

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The class of pentacyclic triterpenes presents a diversity of structures due to the number of rearrangements that the molecule of oxidosqualene undergoes during cyclization, being friedelin the derivative with maximum number of rearrangements. The oxidosqualene cyclases (OSC) are enzymes that catalyze the biosynthesis of triterpenes, generating a wide variety of molecules. This study aims to clone OSC from leaves of *Maytenus ilicifolia* Mart. ex Reissek, a medicinal species from Brazil, for functional analysis of its pentacyclic triterpenes. For this purpose, we adopted the strategy of RT-PCR. Total RNA was extracted from leaves of *M. ilicifolia* and used in the synthesis of cDNA. The PCR amplification of core fragments of OSC genes was performed with partially degenerated primers designed to amplify highly conserved regions among this class of enzymes. The cloned fragments were sequenced and specific primers were designed for a first round amplification of 5′ extremity. BLAST analysis of both round of cloned fragments (~1500 bp) revealed clones with up to 75% similarity with oxidosqualene cyclases already identified, such as beta-amyrin and cycloartenol synthases, which validates the PCR strategy used. The results of sequencing obtained so far cover for ~60% of the whole predicted gene ORF. These results are being used to design new primers for amplification of full length cDNAs by Rapid Amplification of cDNA Ends (RACE), in order to obtain the complete sequences of these oxidosqualene cyclase genes. Future work using functional expression in *Saccharomyces cerevisiae* of the cloned genes will characterize the oxidosqualene cyclases from *M. ilicifolia* leaves. Supported by: FAPESP, CNPQ and PADC.

P03-59
Analysis of the expression of plant specific insert (PSI) domain in the non conventional system Kluveromyces lactis

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Typical plant aspartic proteinases (APs) precursors are characterized by the presence of the *plant specific insert* (PSI) domain of approximately 100 amino acids residues, absent in other APs. The PSI has been implicated in the sorting of APs precursors as well as in the proper folding. The aim of the present work consisted in evaluating the expression of PSI of cirsin, an AP from

Cirsium vulgare (Savi) Ten. (Asteraceae) using the non conventional yeast *Kluveromyces lactis*. To achieve the aim, the a-MF fusion PSI(His)$_6$ protein and its glycosylation putative site mutant, a-MF fusion N86S_PSI(His)$_6$, were generated by cloning both sequences into pKLAC1 vector. In *K. lactis* system, the a-MF domain is processed in the *trans*-Golgi by Kex protease. Simultaneously, the constructs without the a-MF fusion but having the signal peptide, were obtained. Recombinant proteins were expressed in YPD-Gal (4%) medium for 96 hs at 30°C. Product purification was followed by inmunodetection and PMF. In the case of a-MF fusion proteins, PMF analysis and *in vitro* treatment with Kex, showed that a-MF domain was not completely processed inside the *trans*-Golgi. Deglycosylation assays indicated that these proteins were susceptible to PNGase F and Endo H, suggesting proteins were not secreted through the Golgi or at least, they were not nodificated by glycosydases of this organelle. On the other hand, PMF and inmunodetection confirmed that PSI without α-MF was also secreted by the yeast under glycosylated and non glycosylated forms. Glysosylation in these two proteins showed the same susceptibility profile than a-MF fusion proteins. Taken together, these data suggest in this expression system, PSI secretion is not affected by its glycosylation nor follows the regular *trans*-Golgi processing.

P03-60
Plastid thioredoxins f and m in *Arabidopsis thaliana*: localization and functional characterization

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Plants thioredoxins (Trxs) are small and ubiquitous oxidoreductases whose main biochemical role is to produce posttranslational redox modifications on target proteins through a reaction catalyzed by the two conserved cysteines of the active site (WCXPC). Several types of Trxs are localized in the chloroplast: *f*, *m*, *x*, *y* and *z*. Initially, Trxs *f* and *m* were exclusively related with photosynthetic processes, linking the electron transport chain with the carbon assimilation pathway. However, in the last years, these two isoforms have been described to play important roles in other cellular processes such as response to abiotic stress, embryogenesis, growth, transport and protein folding. These additional functions are supported by the localization of Trxs *f* and *m* in non-photosynthetic tissues like seeds, roots and flowers. In this work we search new and specific sites of expression and functions for the plastid Trxs *f1* and *f2* and *m1*, *m2*, *m3* and *m4* of Arabidopsis. The analysis of transgenic *Arabidopsis thaliana* lines containing constructions of the *AtTrx f1, f2, m1, m2, m3 and m4* genes translationally fused to the *GFP* and *uidA* (*GUS*) reporter genes reveals differences in their respective expression patterns. AtTrxs *m2* and *m4* are expressed in roots at early stages of the plant development, AtTrxs *m3* and *m4* in leaves while AtTrxs *f1*, *m2* and *m3* are found in stomata. In order to deep into the search of specific functions we are also analyzing Arabidopsis Trxs *f* and *m* knock-out mutants and their pattern of expression during the plant growth.

P04 – Biochemistry in Medical Diagnosis and Therapy

P04-1
Changes in electrophoretic separation profiles of smelter workers' serum proteins

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Introduction: Occupational exposure to heavy metals such as arsenic, cadmium and lead has already been confirmed by the increased concentration of these metals in smelter workers' blood and urine. Exposure to heavy metals causes the changes in concentration of oxidative stress parameters and can cause damage to the organs. The effect of exposure to heavy metals on the liver metabolism that may be manifested in changes of serum proteins profiles was analysed.
Methods: Protein fractions in smelter workers' serum (albumins, αl-globulins, α2- globulins, β-globulins, γ-globulins) were separated and quantified using capillary electrophoresis PA 800 Plus Protein Characterization System (Beckman Coulter). Proteins were measured by direct absorption at 214 nm.
Results: We observed a decrease in the percentage of α1-globulins in smelter workers' serum exposed to heavy metals in relation to the control group and an increase of β-globulins in smelters' serum. Statistically, no significant changes were found in the percentage of albumin, in serum of occupationally exposed to heavy metals compared with the control group. Exposure to lead causes an increase in the percentage of α2-globulins and a decrease in the percentage of α1-globulins and γ-globulins, what was observed by proportional, direct and inverse correlation, respectively.
Conclusions: The observed abnormities in the protein profile can become important biomarkers in the diagnosis of diseases caused by heavy metals.

P04r-2
Towards new antivirals targeting dengue virus: role of the capsid protein during infection

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Dengue Virus (DV) causes about 20 000 deaths due to viral haemorrhagic fever pathology and infects 50–100 million people every year. No effective treatment is available and several aspects of the viral multiplication and infectivity remain unclear. The function of DV structural proteins, namely the capsid protein (DVCP) were studied in the presence of oligonucleotides and biological membrane models (Large Unilamellar Vesicles) using biophysical techniques (Light scattering, Fluorescence Spectroscopy and Confocal Microscopy) in order to elucidate its role in DV infection. The results obtained reveal that DVCP, so far assigned to structural functions, may play a key role during the entry steps of DV in Cell infection. The biological implications of these results will be discussed, pinpointing novel antiviral selective targets.

P04-3
Cell-based immuno assay for detection of AQP4-Abs in serum of patients with NMO

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Neuromyelitis optica (NMO) is a severe autoimmune demyelinating disease of the central nervous system (CNS) that predominantly affects the spinal cord and optic nerves. The presence of auto antibodies against AQP4 (NMO-IgG or AQP4-Abs) in serum's patients with NMO have significantly improve the diagnosis of this disease and allow critical distinction of this pathology from multiple sclerosis. Here we present a simple method for detection of AQP4-Abs by which the serum of patients is used as the primary antibody in an immunofluorescence assay performed over HEK293 cells stably or transiently transfected with hM23-AQP4 C-terminally EGFP tagged. After 24 hour of transfection cells were fixed, permeabilized and blocked with FCS and BSA. Two protocols were compared one in which the primary antibody was added immediately after the blocking step follow by the regular washing steps and incubation with the secondary Ab, and other in which cells were kept frozen for variable time in blocking solution with 10%DMSO before adding the primary Ab. We have analyzed a total of 68 patient's serum diagnose either as NMO (8), multiple sclerosis (36), neuritis optica (4), longitudinally extensive myelitis (6), recurrent myelitis (2), undiagnosed (7), and healthy donors (5). Qualitative analysis by fluorescence microscopy revealed similar results from both protocols, and quantification of fluorescence signal with the image-J software confirm comparable values in both. Our method for detection of NMO-IgG revealed the presence of anti-AQP4 Abs in the total of serums from patients clinically diagnosed as NMO and the absence of it in all the rest of serums analyzed. We confirmed that sensitivity and specificity of both protocols used are extremely high (∼100%) for detection of AQP4-Abs; and demonstrated that NMO-IgG detection can be done in just one day of analysis since frozen cells kept ready to incubate with the serum produce strong fluorescence signal.

P04-4
The role of HPV E6 proteins in cellular transformation

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Cancer is a leading cause of death worldwide and accounted for 7.4 million deaths or 13% of all deaths in 2004 (WHO). Approxi-

mately 500 000 of these deaths are due to oesophageal cancer alone, one of the major cancers in Eastern and Southern Africa. Several studies have shown HPV11 DNA to be integrated in nearly 40% of oesophageal tumors and present in only 3% of normal healthy asymptomatic individuals, implicating it as a possible risk factor. This study compared the roles of the E6 proteins from HPV11 (low-risk) and HPV18 (high-risk) and evaluated their effects on the gene expression profile. In order to identify genes required for initiation of transformation, the E6 genes were cloned into an adenoviral vector for transformation of normal EPC2 oesophageal epithelial cells to induce intense, high-level expression of these genes in these cells. RNA isolated from these cells was used in cDNA microarray analysis to study and compare the effects that the E6 genes have on endogenous gene expression patterns during the early stages of transformation. Microarray study is currently being carried out for the analysis of important genes. Signaling pathways involved in E6-mediated transformation will be identified, and the effect of inhibiting these pathways will be investigated. This research will aid in the identification of host target genes in HPV E6 mediated cellular transformation, and help elucidate possible roles of low-risk HPVs in oesophageal cancer. If low-risk HPVs do play an active role in the development of cancer, it would be an important breakthrough in not only the study of oesophageal cancer, but other cancers where HPVs are known to be involved.

P04-5
Identification of peptide aptamers as specific inhibitors of snail proteins

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The Snail family of transcription factors have been characterized as potent inducers of the epithelial to mesenchymal transition (EMT), a key developmental program that is often activated during cancer invasion and metastasis. Furthermore, they could be considered as ideal target for a 'smart' cancer therapy because of their absence in normal adult tissues and their ability to confer invasive capacity and cell death resistance to the cells that reactivate them. With this in mind, we employed the Y2H technique to screen a combinatorial peptide library and identify small peptides that selectively interact with Snail2 and could modulate its oncogenic functions. After the yeast primary screening of the library and secondary confirmation by Co-Affinity Purification assay in a tumour breast cell line, 10 PAs have been identified as specific interactors of human Snail2 protein.

At the same time, we tested whether the PAs could interfere with the ability of Snail proteins in repressing the *Ecadherin* promoter, one of their main functions, crucial in tumor progression. So far, 2 PAs clearly interfered with this function in a Luciferase Assay in tumour cells. Finally, we wanted to assess whether synthetic peptides corresponding to the PAs sequences could be used as 'small-molecule' targeted inhibitors of Snail function. To this aim, we designed 1 synthetic peptide conjugated with fluorescein and it has been administered to cell cultures alone and in combination with a red-peptide carrier (Cy5.5-iRGD) to unveil a role in influencing Snail oncogenic functions. Results of the interactions tested and preliminary analysis the PAs biological relevance will be presented and discussed.

P04-6
Immunomodulatory action of new curcumin analogue (BDMC33) on major pro-inflammatory mediator expression in cellular model of rheumatoid arthritis

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A series of curcumin analogues were synthesized and screened for anti-inflammatory activities by evaluating the nitric oxide (NO) inhibitory activity upon activated macrophages *in vitro*. The preliminary screening results have demonstrated that BDMC33 [2,6-bis(2,5-dimethoxybenzylidene) cyclohexanone] exerted promising and improved anti-inflammatory activity as compared with curcumin. The anti-inflammatory properties of BDMC33 and its underlying mechanism action were elucidated upon two cellular systems which are IFN-γ/LPS-stimulated macrophages (RAW264.7) and PMA-stimulated synovial fibroblast (HIG-82). BDMC33 significantly inhibited the IFN-γ/LPS-induced secretion of major pro-inflammatory mediators of macrophages in dose-dependent manner, includes NO, PGE_2, TNF-α and IL-1β. The inhibitory action of BDMC33 was mediated via attenuation of IKK phosphorylation as well as suppression of ERK1/2 and JNK1/2 phosphorylation, but not p38 MAPK. In addition, BDMC33 significantly suppressed the PMA-induced production of matrix metalloproteinase (MMP) in synovial fibroblast, includes MMP-1, MMP-3 and MMP-9 as well as the inflammatory gene expression of COX-2 and IL-6. The underlying mechanism of BDMC33 on synovial fibroblast was also mediated via NF-κB signaling pathway; as p65 NF-κB nuclear translocation and NF-kB DNA binding activity were being attenuated. Collectively, the experimental data suggests that the immunomodulatory action of BDMC33 is attributed through attenuation of pro-inflammatory mediator production and MMPs of macrophages and synovial fibroblast cells, which could represents a promising lead compound for future therapeutic development in rheumatoid arthritis management.

P04-7
Melanin may contribute to fitness of *Acinetobacter baumannii*

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Acinetobacter baumannii causes a variety of nosocomial infections, including bloodstream infections, ventilator-acquired pneumonia, meningitis, urinary tract infections, and wound infections, resulting in mortality rates ranging from 26 to 68%. Due to the multi-drug resistance, treatment of its infection has been extremely difficult. Little is known about the virulence factors of this organism. Melanin, that can increase the survival of microorganisms by protecting DNA and other molecules from UV damage, has been implicated as a virulence factor as well as important to microbial pathogenesis. In this study, a mini-Tn10 transposon mutant library of *A. baumannii* ATCC 17978 was constructed. One of the mutants, YHL1037, produced a black pigment on solid medium which was identified to be melanin by its character of Fourier transform infrared spectroscopy analysis. To analyze the transposon insertion site, inverse PCR was performed and the amplicon obtained was sequenced. Sequence comparison indicated that YHL1037 had a Tn10 inserted in locus A1S_3416, encoding homogentisate 1,2-dixoygenase gene (hmgA). HmgA,

involved in tyrosine metabolism is known to degrade homogentisic acid (HGA) to 4-maleyl-acetoacetate; in its absence, HGA is more readily oxidized to benzoquinoneacetic acid, which is then polymerized to form pyomelanin. In complementation test, the phenotype of melanin hyperproduction was reverted back to the non-pigmented wild-type, suggesting that hmgA disruption is indeed responsible for the hyperproduction of pyomelanin in *A. baumannii* ATCC 17978. Moreover, the hmgA mutant resulted in increased toxicity of *A. baumannii*, causing higher mortality rates than the parental strain in rat-bacteremia model. In addition, UV sensitivity and H2O2tolerance assay showedthat the hmgAmutant was able to tolerate higher doses of UV irradiation and higher concentrations of H2O2 than the parental strain. Collectively, these results indicate that melanin is a virulence factor of *A. baumannii* and its hyperproduction can increase tolerance to strict environments.

P04-8
Cellular effects of novel dinuclear platinium(II) complexes in human breast cancer cells

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Polynuclear platinum complexes constitute a novel class of prospective anticancer agents that have shown some peculiar activities as compared with mononuclear platinum compounds. Structurally novel platinum complexes that bind to DNA differently than cisplatin may have distinct cytotoxicity and side effect profiles. The present study was undertaken to extend our recent findings related to the antineoplastic activity of novel dinuclear platinum(II) complexes with berenil and amine ligands. The effects of $Pt_2(amine)_4(berenil)_2$ on collagen biosynthesis, $beta_1$-integrin receptor, IGF-I receptor and the expression of several proteins in the signal generated through the receptors like: phosphorylated MAP-kinases (ERK1/2 and p38), phospho Akt, NF-kB and the presence of apoptosis in human breast cancer cells were compared to those caused by cisplatin. The up regulation of $beta_1$-integrin and insulin-like growth factor I (IGF-I) receptor expression by the complex was shown to be accompanied by an increase in the expression of mitogen activated protein kinases in breast cell lines. The phenomenon was related to the increased expression of nuclear factor-kappaB (NF-kB) by these compounds as shown by the Western immunoblot analysis. To determine the nature of cell death induced by these compounds in human MDA-MB-231 and MCF-7 breast cancer cells, we measured cell death by flow cytometric analysis after annexin V-FITC and propidium iodide staining. We also determined change of the mitochondrial membrane potential, caspase-3 activity and DNA degradation. These results demonstrate that dinuclear platinum(II) compounds treatment activate a mitochondria-mediated apoptotic pathway.

P04-9
Effects of melatonin on the glutathione system in the blood of alloxan diabetic rats

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Alloxan diabetes was reported to induce oxidative stress and generates ROS. Melatonin is known to be involved in antioxidant defence, and the aim was to determine the influence of melatonin on basal levels of glucose, redused glutathione (GSH), activity of glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx), and glutathione reductase (GR) in the blood of alloxan diabetic rats.

The study involved 67 male white rats weighting 180–200 g. Alloxan diabetes was evoked via single injecting the rats with 5% alloxan monohydrate solution in the dose of 170 mg/kg. The animals were divided into groups: I – intact animals, II – alloxan diabetic rats with overt (basal glyacemia >8.0 M) and latent glyceamia (basal glyacemia <6.9 M) diabetes, III – animals with overt and latent diabetes were introduced melatonin intraperitoneally in the dose of 10 mg/kg at 8 a.m. daily during 6 weeks.

The introduction of melatonin promoted normalization of the level of basal glycemia in diabetic rats indicating hypoglycemic action of melatonin administration. In the blood of rats with overt diabetes GSH content decreased on 31%, while in animals with latent diabetes this index increased on 28% as compared with intact animals. Activity of G6PD, GPx and GR in the blood of rats with overt diabetes was on 25%, 22%, and 20% respectively lower than in intact rats. Increased content of GSH in rats with latent diabetes is probably induced by enhanced regeneration of the oxidized form. In the blood of rats of this group activity of G6PD, GPx and GR was on 30%, 27%, and 23% respectively higher than in intact ones.In the blood of alloxan diabetic rats receiving melatonin the indices mentioned above didn't differ from the control group.

Under conditions of overt diabetes exogenous melatonin activates glutathione dependent enzymes in the blood of alloxan diabetic rats that ultimatemly provides increased content of GSH – one of the main endogenous antioxidant.

P04-10
Liposome-entrapped immunodominant peptides of the myelin basic protein suppress EAE in DA rats by downregulation of Th1-cytokines and induction of BDNF production in CNS

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Multiple sclerosis (MS) is a severe inflammatory and neurodegenerative disease of an autoimmune background. Besides the existence of well-known therapeutics, the development of novel approaches for MS treatment is of a high importance in the modern pharmacy. We report here immunodominant peptides of myelin basic protein (MBP) encapsulated in small unilamellar mannosylated liposomes (mSUV) as an effective composition for treatment of experimental autoimmune encephalomyelitis (EAE) in DA rats. Liposome-entrapped MBP_{46-62} was the most effective in decreasing of the maximal disease score during the first attack, whereas $MBP_{124-139}$ and $MBP_{147-170}$ prevented the development of the exacerbation stage. Administration of a mixture of determined immunodominant MBP peptides, encapsulated into the mannosylated liposomes, significantly suppress the protracted EAE by preventing production of autoantibodies, downregulation of Th1 cytokines and induction of brain-derived neurotrophic factor (BDNF) production in central nerve system. Synergistic liposome-mediated effect of MBP peptides decreases overall disease course with the moderate first attack and fast outcome from the exacerbation, suggesting a novel therapeutic modality for MS treatment.

P04-11
Inhibition of MAPK and PI3K signalings involved in lipopolysachharide-induced matrix metalloproteinase-9 expression by macelignan in rat chondrocyte explants

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Gout is caused by the accumulation of uric acid in connective and joint tissues that lead to inflammation and over-expression of pro-inflammatory molecules including matrix metalloproteinase (MMP)-9. We investigated the inhibitory effects of macelignan isolated from *Myristica fragrans* Houtt. on the expression of MMP-9 protein and gene in rat chondrocyte explants stimulated by lipopolysaccharide (LPS) by performing Western blot, gelatin zymography, and RT-PCR. We also determined if macelignan interfered the signaling pathways, i.e. MAPK and PI3K/Akt, mediated LPS-stimulated MMP-9 gene expression in chondrocytes. Our results demonstrated that LPS induced MMP-9 expression, and macelignan dose-dependently inhibited the expression of MMP-9 secretion, protein and gene in LPS-induced chondrocytes. Macelignan suppressed phosphorylation of ERK1/2, p38, JNK1/2 and Akt involved in LPS-induced MMP-9 gene expression in chondocytes. These results suggest that macelignan decreases MMP-9 gene expression via blocking MAPK and PI3K signaling pathways. Further *in vivo* study is needed to determine the exact chondroprotective potential of macelignan.

P04-12
Cytotoxicity and topoisomerases I and II inhibition study of Pt-berenil complexes

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Berenil can exhibit intercalative, as well as minor groove binding, properties when it binds to both DNA and RNA duplexes, while also exhibiting a preference for DNA duplexes with unobstructed minor grooves. Berenil preferentially recognizes and binds to AT-rich DNA sequences and it is strong catalytic inhibitor of mammalian DNA topoisomerases. We have synthesized of several Pt-berenil complexes. We are expecting that these complexes would localize in the vicinity of the DNA, and the combined effect resulting from platination and minor groove binding might confer cytotoxic activity to these complexes. Cell viability of breast cancer cells was measured by the method of Carmichaela using tetrazolium salt. These compounds are approximately 10 times more potent than cisplatin. The binding affinities of Pt-berenil compoundsto calf thymus DNA, T4 coliphage DNA, poly(dA-dT)$_2$ and poly(dG-dC)$_2$ were compared by using the ethidium displacement assay. The large apparent binding constants for T4 coliphage DNA for these compoundsgave evidence of their minor-groove selectivity, because the major groove of T4 coliphage DNA is blocked by α-glycosylation of the 5-(hydroxymethyl)cytidine residues. The binding constants obtained for binding of these compounds to poly(dC-dG)$_2$ polymer are almost 30 smaller than the association constants for binding to poly(dA-dT)$_2$. The ability of Pt-berenil compounds to inhibit topoisomerase I and II activity was quantified by measuring the action on supercoiled pBR322 DNA substrate as a function of increasing concentration of the ligands by the use of agarose gel electrophoresis. These results demonstrated that Pt-berenil compounds have topoisomerase II inhibitory activity with 50% inhibitory concentrations (IC_{50}) ranging from 5 to 50 μM.

P04-13
NMR studies of a novel anticancer metallo-drug with proteins and oligonucleotides

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In the last decade, the research in anticancer drugs based on several metal ions different to platinum has been extensively developed (1). Among these, gold, iridium, rhodium, osmium, and ruthenium are the metals most commonly used. Our group is synthesizing different metallo-drugs, highly efficient against determined cancer cell lines, as well as characterizing their mechanism of action versus their possible biomacromolecule targets, being these not only nucleic acids, but also proteins. In fact, Ru(II) complexes are believe to interact mainly (contrary to Pt(II) drugs) with proteins.

We have previously described the synthesis and spectroscopic characterization of the Ru(II) complex [(|^6-*p*-cymene)Ru(N^C)Cl] (HN^C = 9-aminoacridine) (2). Its IC_{50} value against T47D cell lines is 3.7 lower than that of cisplatin.

Now, we are studying the interaction of this Ru(II) complex with model proteins (ubiquitin, rusticyanin) and small oligonucleotides by several spectroscopies, specially by Nuclear Magnetic Resonance. ^{1}H,^{15}N heteronuclear NMR permits allocate the specific protein residues that interacts with the complex and thus is a first preliminary step to know how the drug can interact with protein targets.

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P04-14
Calcitriol and imatinib affect the level of expression of molecular markers of stemness by colon cancer cells pre-selected with 5-Fluorouracil

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Expression of CD133, LGR5, CD44, EphB4 known as molecular markers of cancer stem cells was analysed in 5-Fluorouracil (5-FU)-preselected human colon cancer cell line HT29 prior and after the exposure to imatinib or calcitriol used as differentiation-inducing agents. HT29 cells express C-kit as a target for imatinib and VDR as a receptor for calcitriol. Following the exposure to conventional cytostatic drug 5-FU for 24 hour and additional incubation in the absence of 5-FU for 3 days, preselected HT29 cells were passaged and cultured for 4 days with or without imatinib or calcitriol. CD133+ cell fraction of 5-FU-preselected cells which was obtained by magnetic separation demonstrated higher growth and clonogenic potential than CD133$^-$ cell frac-

tion. By flow cytometry, percentage of AC133/CD133+ cells in HT-29 cell line gradually decreased after 5-FU treatment and after passage and 4-day culture. Except for *EPHB4*, by real-time PCR method relative mRNA expression of CSC-related genes *CD133*, *LGR5*, *CD44* decreased on day 3 after 5-FU treatment. After passage and 4-day culture of 5-FU preselected cells, the level of mRNA expression of colon CSC-related genes, i.e. *CD133*, *LGR5* and *CD44* increased. Such increase of *CD133* and *LGR5* mRNA expression was inhibited in the cultures set in the presence of differentiation-inducing agents. On day 4th after passage of 5-FU preselected cells, mRNA expression of differentiation marker *VIL1* increased twice if cultures were set in the absence of differentiation-inducing factors. The mRNA expression of *VIL1* increased more than 3-times in the cultures set with imatinib or calcitriol. Our data show that calcitriol and imatinib promote differentiation of the 5-FU-preselected HT-29 cells which express markers of stemness. MNiSW grant NN402139738.

P04-15
In vivo effect of epilobium hirsutum on xenobiotic metabolising CYP2E1, CYP1A1, glutathione peroxidase and NAD(P)H:quinone oxidoreductase 1 enzymes in rat liver

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Epilobium hirsutum L. (EH) one of the members of Onagraceae family is known as its analgesic, anti-microbial and anti-proliferative activity, and used as an alternative medicine. CYP2E1 and CYP1A1 known as xenobiotic metabolizing enzymes, serve as a metabolic activation route that yields reactive metabolites which initiate toxic and carcinogenic events and responsible for the bioactivation of environmental carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons (PAHs). Hazardous effects of ROS can be eliminated by the action of *Glutathione peroxidase (*GPx) and NAD(P)H:quinone oxidoreductase 1 (NQO1). In the present study we aimed to investigate the effect of water extract of EH on CYP2E1, CYP1A1, GPx and NQO1 mRNA and protein expressions, as well as the activities towards specific substrates. Identification of phenolic compounds was done by using liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS). 19 mg extract/kg body weight/day was injected intraperitoneally to male rats for 9 days. Enzyme activity studies have shown that EH extract inhibited the CYP2E1 dependent aniline 4-hydroxylase (80%) and NDMA N-demethylase (20%) and CYP1A1 dependent ethoxyresorufin-O-deethylase (32%) activities. On the other hand, GPx and NQO1 activities were stimulated 1.6 fold and 3.6 fold, respectively. Effects of EH on protein expression were analyzed by Western blotting and results showed that the CYP2E1, CYP1A1 protein levels were decreased while that of GPx and NQO1 increased. The alteration in mRNA expression was investigated by qRT-PCR. EH treatment of animals caused lower expression of mRNA for CYP2E1, CYP1A1, and higher mRNA synthesis for GPx and NQO1. These results point out that EH may change the metabolism of a number of chemicals including carcinogens by modulating the enzymes involved in xenobiotics activation-detoxification pathways.This work is supported by TUBITAK, Project No: 109R012, Turkey.
Keywords: *Epilobium hirsutum* L., CYP2E1, CYP1A1, GPx, NQO1, protein/mRNA expression, Xenobiotic Metabolism

P04-16
Characterization of monoclonal antibodies against glutamate carboxypeptidase II, the diagnostic and potential therapeutic prostate cancer marker, via the method of surface plasmon resonance

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Glutamate Carboxypeptidase II (GCPII) is one of the most promising target for diagnostic and therapy of protate cancer patients. To this date, just one antibody (ProstaScint^{TM}) has made it through to practical use in diagnostics, but many other antibodies, or its fragments, are intensively studied. Even though the reservoir of antibodies seems to be wide, their characterization often restricts only to their sensitivity towards GCPII.

In this study, we investigate several antibodies (7E11, 3C6, J591, J415, Y-PSMA-1, Y-PSMA-2, 3E6, GCP-02, GCP-04, GCP-05) and test them for their affinity towards native and denature forms of human GCPII and also its close homologs via the method of surface plasmon resonance.We observed specific binding to native GCPII by antibodies GCP-05, 3C6, J591, J415 and denaturated GCPII by antibodies Y-PSMA-1, Y-PSMA-2, 3E6, GCP-04, GCP-02. We were able to compare binding affinities of these antibodies and characterize their binding kinetics. Aditionally, we were able to characterize kinetic of binding of antibody 7E11 towards the synthesized peptide which mimics the intracellular portion of human GCPII. We also characterized the binding specificity of all antibodies towards GCPII close homolgs showing that, except antibodies GCP-02 and GCP-04, all antibodies are highly specific to extracellular portion of human GCPII.We hope that this study will shed light on the pool of available antibodies against GCPII and helps the researches to choose the best candidates for primary research, clinical trials, or commercial use.

P04-17
Different sensitivity on electrochemotherapy of human cell lines *in vitro*

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Introduction: The application of the cell membrane electroporation in combination with cytotoxic drugs could increase their transport into cells. The combination of electroporation and application of drugs with inhibited transport is known as electrochemotherapy (ECT). Permeabilization cell membrane depends on pulse duration, pulse amplitude and the numbers of pulses delivered.

Materials and Methods: The effect of electroporation with and without drug (bleomycin) was performed by analysis of cells viability (MTT assay). We investigated human breast adenocarcinoma (MCF-7/WT) and its doxorubicin resistant (MCF-7/DOX), on human lung carcinoma (A549), ovarian adenocarcinoma (SKOV3), on human gastric carcinoma parental (EPG85-257P) and its daunorubicin resistant (EPG-85-257RDB) and normal cell line human keratinocyte (HaCaT). Bleomycin was used at 30 nM concentration. The electroporation parameters were: 100, 500, 1000 V/cm, 100 μs, eight impulses. As electrodes we used thin stainless-steel parallel plates (4 mm gap). The MTT assay (Sigma)

was used to test the mitochondrial metabolic function (24 hour, 72 hour and 120 hour after electroporation).

Results: Electroporation in combination with bleomycin efficiently decreased cells proliferation simultaneously with increasing voltage. In MTT test we obtained similar results. As was observed electroporation did not significantly reduce cells viability. Resistant cells were equally sensitive to electrochemotherapy with drugs as their sensitive counterparts.

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P04-18
Anti-angiogenic and anti-tumor properties of in silico-designed peptides from CD47

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CD47 is a ubiquitously expressed membrane receptor implicated in many pathophysiological processes including apoptosis, cell proliferation, inflammation and cardiovascular responses. CD47 and its endogenous agonist thrombospondin-1 (TSP-1) might therefore represent key targets in tumor microenvironment for development of innovative therapeutic strategies against tumorigenesis and metastasis. Molecular modeling studies led to identify two CD47-derived peptides (IESQLLKGDAS and its disulfide-bound analogue CEVSQLLKGDAC) acting as putative antagonists of TSP-1/CD47 interaction, as confirmed by co-immunoprecipitation and ELISA binding experiments. *In vitro*, a two-fold diminution in 2D and 3D endothelial cells migration was observed in the presence of 100 μM peptide, suggesting anti-angiogenic properties. Consistent with this, HUVECs tube formation and *ex vivo* Matrigel-induced angiogenesis on mouse aortic rings explants were also inhibited in the presence of CD47-derived peptides. In addition, disruption of VEGFR signaling was observed on endothelial cells under peptide treatment. Finally, CEVSQLLKGDAC cyclopeptide induced *in vivo* tumor necrosis and disturbed tumor vascularization on an allograft model of murine B16F1 melanoma cells, as shown by HPS staining, CD31 immunostaining, MNR micro-imaging and CT-scan angiography of tumors. Finally, these bioinformatically-modeled TSP-1/CD47 antagonist peptides exhibit strong anti-angiogenic activities *in vitro*, *ex vivo* and *in vivo* and could therefore represent new exciting tools for cancer treatment.

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P04-19
Synthesis and effect of a polyamine analogue of chloramphenicol on peptide bond formation

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Chloramphenicol (CAM) is a broad spectrum antibiotic, which inhibits protein synthesis in prokaryotes interacting with the large ribosomal subunit. Previous studies in our laboratory have demonstrated that polyamines influence the binding of CAM to the ribosome. These observations prompted us to synthesize an analogue of CAM harboring one spermidine (SPD) unit, which replaces its dichloro-methyl edge, and to test its ability to inhibit peptide bond formation in *Escherichia coli* ribosomes. In the present study, the inhibitory effect of the CAM-SPD conjugate was tested by detailed kinetic analysis in an *E. coli* cell-free system, in which a peptide bond is formed between puromycin and AcPhe-tRNA bound to the P-site of poly(U)-programmed ribosomes.

The conjugation of spermidine with CAM did not alter the type of inhibition, previously observed for the parent compound; CAM-SPD was found again to interact with ribosomes via a two-step mechanism, behaving as a slow-binding competitive inhibitor of peptide bond formation. Namely, CAM-SPD (I) reacts rapidly with the initiator ribosomal complex (C) to form an encounter complex CI, which is then isomerized slowly to a tighter complex, C*I. The overall inhibitory activity of CAM-SPD was found approximately three-fold higher (K^*_i = 0.28 μM), compared to that of CAM (K^*_i = 0.88 μM).

Our results show that conjugation of SPD to CAM facilitates the binding of CAM to the ribosome, probably by creating a bridge connecting CAM with the ribosome. Alternatively, the presence of a polyamine molecule deep in the heart of the catalytic center of peptidyl transferase may induce changes in the ribosomal conformation which favors CAM binding.

P04-20
Antioxidative and antiapoptotic genes expression in irradiated leukemia cells upon fullerenol nanoparticle treatment

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Recent data established the prospective applications for fullerenol ($C_{60}(OH)_{24}$) nanoparticle (FNP) in many fields, such as antioxidants, neuroprotective agents, and potential anti-radiation drugs. Leukemia cell sensitization to apoptosis induced by ionizing radiation is achieved by upregulation of ROS production and/or downregulation of antioxidative enzymes. Therefore, our aim was to analyze the potential role of fullerenol nanoparticle in modulation of the leukemic cellular response to irradiation. We used the qRT-PCR to analyze the expression level of mRNA for 11 genes in irradiated (IR) and FNP pre-treated irradiated cells (FNP + IR) K562 cells, and compared gene expression level with the overall cell survival, determined by DET and MTT assays. Interesting result of similar percentage of cell survival in FNP and FNP + IR groups (67.9% versus 63%), indicated the possible protective effect of FNP in irradiated cells. Under oxidative stress induced by radiation, cytoprotective genes such as GSTA4, MnSOD, NOS, CAT and HO-1 were upregulated in FNP pre-treated K562 cells in comparison with control group, IR and FNP – treated non-irradiated cells. Amongst the antioxidative enzymes, only the mRNA of Cu, Zn – SOD (SOD-1) was down-regulated, while gGT, GPX and GSTP1 were not significantly modulated in FNP-treated irradiated cells. Together with survival data and significant overexpression of anti-apoptotic Bcl-2 and

Bcl-xL genes, our results may indicate that FNP exerts cytoprotective function in K562 leukemic cells, rendering K562 cells more tolerant to radiotherapy.

P04r-21
Anti-angiogenic properties of carnosol and carnosic acid, two major dietary compounds from rosemary

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The use of rosemary (Rosmarinus officinalis) leaves and their constituents as a source of dietary antioxidants and flavoring agents is continuously growing. Carnosol and carnosic acid, two major components of rosemary extracts, have shown activity for cancer prevention and therapy. We investigate the cytotoxic and anti-angiogenic activities of carnosol and carnosic acid, in order to get further insight into their mechanism of action. Our results demonstrate that the mentioned diterpenes inhibit certain functions of endothelial cells, namely, differentiation, proliferation, migration and proteolytic capability. Our data indicate that their growth inhibitory effect, exerted on proliferative endothelial and tumor cells, could be due to, at least in part, an induction of apoptosis. Inhibition of the mentioned essential steps of *in vitro* angiogenesis agrees with the observed inhibition of the *in vivo* angiogenesis, substantiated by using the chick chorioallantoic membrane assay.

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P04-22
Survival, classifications, and desmosomal plaque genes in non-small cell lung cancer

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Novel biomarkers are required to improve prognostic predictions obtained with lung cancer staging systems. This study of 62 surgically-treated Non-Small Cell Lung Cancer (NSCLC) patients had two objectives: (i) to compare the predictive value of T-stage classifications between the 6th and 7th editions of the Tumor, Node and Metastasis staging system (TNM); and (ii) to examine the association of Pkp1 and/or Krt15 gene expression with survival and outcomes. Multivariate Kaplan-Meier survival analyses were performed, examining the relationship of survival with tumor size, recurrence, and stage (by each TNM edition) and with the single/combined expression of Pkp1 and/or Krt15 genes. Five-year survival rates only significantly differed as a function of tumor size in patients without recurrence when estimated using the 6th edition of the TNM classification and only in patients in pathologic stage IA using the 7th. Overall survival (OS) for patients with elevated expression of both genes was 13.5 months in those with adenocarcinoma and 34.6 months in those with squamous cell carcinoma. OS was 32.7 months in patients with Pkp1 gene upregulation and 30.9 months in those with Krt15 gene upregulation. In conclusion, survival estimations differed between TNM editions 6 and 7 (T-staging) and according to Pkp1 and/or Krt15 gene expression.

P04-23
4-Methylumbelliferone is an Effective Inhibitor of *in vitro* and *in vivo* Angiogenesis

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4-Methylumbelliferone (4-MU) is an hyaluronic acid biosynthesis inhibitor. The objective of the present study was to determine the potential of 4-MU as an anti-angiogenic compound. To fulfil this aim, we used cultured endothelial cells to perform an array *of in vitro* assays, as well as two different *in vivo* angiogenesis assays. Here, we demonstrate that, in fact, 4-MU behaves as a new inhibitor of both *in vitro* and *in vivo* angiogenesis that affects several key steps of angiogenesis, including endothelial cell proliferation, adhesion, tube formation and extracellular matrix remodeling. Taken together, our results suggest that 4-MU may have potential as a new candidate, multi-targeted bio-active compound for anti-angiogenic therapy. [Our experimental work is supported by grants PS09/02216 (Spanish Ministry of Science and Innovation, ISCIII and FEDER), and CVI-6585 and funds from group BIO-267 (Andalusian Government and FEDER). The 'CIBER de Enfermedades Raras' is an initiative from the ISCIII (Spain)].

P04-24
Increased complement C3 and adipsin protein expression in human coronary atherosclerotic plaques are related to smoking status

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Little information available about any link between smoking status and protein expression levels of acylation stimulating protein (ASP), complement 3 (C3) and adipsin in coronary atherosclerotic plaque. We aimed to determine protein expression levels of ASP, C3 and adipsin by Western blot analysis in the plaques obtained from coronary artery bypass grafting (CABG) and to evaluate whether there is any relationship with smoking status. Coronary artery specimens were obtained from 32 consecutive patients (26 men and 6 women) who underwent CABG procedure in Cardiovascular Surgery Department of Gülhane Military Medical Academy Hospital. Smoking was classified as current smoking [smoking more than five cigarettes within the past 3 months (n = 7)], smoked in the past [>3 months and

< 40 years (n = 15)], or never smoking (n = 10). Increased C3 and adipsin protein expression was observed in both smoked in the past and currently smoker patients than never smoked. We showed that no signal of protein expression of ASP in the plaques in both groups. In conclusion, this is the first report regarding the association of C3 and adipsin, but not ASP, with smoking status in coronary atherosclerotic plaque. Our results provide confirmatory data to prior publications dealing with structural changes in C3 and adipsin induced by smoking, in addition to other physiopathological mechanisms, might accelerate the atherosclerotic process. As the present data provide evidence of association rather than of causation, the biological significance of associations must be interpreted with great caution.

P04-25
Statins inhibit ABCB1 and ABCG2 transporters activity and increase antileukemic potency of imatinib *in vitro*

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Although treatment of chronic myeloid leukemia (CML) was revolutionized by the introduction of imatinib, drug resistance is still a significant problem. In some patients, changes of intracellular concentration of imatinib may influence its therapeutic efficacy. Imatinib concentration is established in result of drug influx (mediated by OCT-1) and efflux (mediated by ATP-binding casette transporters: ABCB1 and ABCG2). As modulation of membrane cholesterol level may conformationally change the activity of ABC transporters, we decided to evaluate effect of HMGCoA inhibitors (statins) on imatinib activity.

As measured using specific substrates statins significantly inhibit efflux capacity of ABCB1 and ABCG2. The effect is completely reversed by the addition of cholesterol. Moreover, addition of statins caused 2–3-fold increase in intracellular concentration of radiolabeled 14C-imatinib in CML cell lines and in primary CML CD34+ cells (incl. clones resistant to imatinib). Statins did not influence initial concentration of imatinib, suggesting that their effects are not mediated by influx activity changes. Cytotoxic assays revealed that statins synergistically enhance imatinib cytotoxicity both in cell lines and in primary CML CD34+ cells. Combination of imatinib and statins induced cell cycle arrest and increased percentage of apoptotic cells. Statins also reduced imatinib-induced phosphorylation of the adaptor protein CrkL.

We conclude that statins increase therapeutic efficacy of imatinib through the inhibition of ABCB1 and ABCG2. Statin-mediated depletion of cholesterol changes conformational status of the pumps, and in that way modulate intracellular concentration of the drug. The addition of statins may become attractive treatment modality for the selected CML patients.

P04-26
Functional characterization of splicing and ligand-binding domain variants in the LDL receptor

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder mostly caused by mutations in the *LDLR* gene. Although the detection of functional mutations in the *LDLR* gene provides an unequivocal diagnosis of the FH condition, there are many variants whose pathogenicity is still unknown. The aims of this study were to set up a rapid method to determine the effect of *LDLR* mutations, thereby providing an accurate diagnosis of FH, and to functionally characterize six *LDLR* mutations detected at high frequency by the LIPOchip® platform (Progenika Biopharma, Spain) in the Spanish population. LDLR expression and activity were analyzed by one-single-step flow cytometry assay and confocal microscopy. Splicing effects were determined by sequencing reverse transcription polymerase chain reaction products. The analysis of three heterozygous variants with a single point mutation within the low-density lipoprotein binding domain allowed us to classify the c.806G > A variant as nonpathogenic, and c.862G > A and c.895G > A variants as causative of FH. The results obtained for three variants affecting donor splice sites of the LDLR mRNA, c.313 + 2dupT, c.1186 + 5G > A, and c.1845 + 1G > C, demonstrated that these mutations are pathogenic. These results expand our knowledge of mutations responsible for FH, providing an accurate diagnosis and leading to early treatment to reduce the risk of premature cardiovascular events.

P04-27
Functional characterization of splicing and ligand-binding domain variants in the LDL receptor

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder mostly caused by mutations in the L*DLR* gene. Although the detection of functional mutations in the *LDLR* gene provides an unequivocal diagnosis of the FH condition, there are many variants whose pathogenicity is still unknown. The aims of this study were to set up a rapid method to determine the effect of *LDLR* mutations, thereby providing an accu-

rate diagnosis of FH, and to functionally characterize six *LDLR* mutations detected at high frequency by the LIPOchip® platform (Progenika Biopharma, Spain) in the Spanish population. LDLR expression and activity were analyzed by one-single-step flow cytometry assay and confocal microscopy. Splicing effects were determined by sequencing reverse transcription polymerase chain reaction products. The analysis of three heterozygous variants with a single point mutation within the low-density lipoprotein binding domain allowed us to classify the c.806G > A variant as nonpathogenic, and c.862G > A and c.895G > A variants as causative of FH. The results obtained for three variants affecting donor splice sites of the LDLR mRNA, c.313 + 2dupT, c.1186 + 5G > A, and c.1845 + 1G > C, demonstrated that these mutations are pathogenic. These results expand our knowledge of mutations responsible for FH, providing an accurate diagnosis and leading to early treatment to reduce the risk of premature cardiovascular events.

P04-28
Arginine deprivation and canavanine treatment: a new potent combinational anticancer approach

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We revealed that in 2D monolayer culture human epithelial cancer cells differed in the level of sensitivity to arginine depletion and p53-mediated cell cycle regulation might be responsible for this disparity. At the same time, we showed that in 3D spheroid culture tested cells were more resistant to arginine depletion. Enhanced resistance of spheroids to arginine withdrawal warrants the search for approaches that in combination with arginine starvation will accelerate its antineoplastic potency. We demonstrated that combination of arginine deprivation with low dose of arginine natural analogue canavanine efficiently and selectively reduced malignant cell growth, survival and recovery by accelerating caspase-mediated apoptosis in monolayer and spheroid cultures. We also revealed that pre-incubation of spheroids under arginine-depleted culture conditions resulted in a 1.5-time increase in sensitivity to external irradiation. Radiosensitivity increased by a factor of 3 when arginine deprivation was combined with canavanine treatment. These data suggest that arginine deficiency alone or in combination with chemotherapeutical approaches is a promising strategy to sensitize cancer cells to irradiation.

P04-29
Novel 7630-base pair mitochondrial DNA deletion in a Mexican patient having Kearns-Sayre Syndrome

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Mitochondrial DNA (mtDNA) mutations have been associated with different illnesses in humans including Kearns-Sayre syndrome (KSS) associated with deletions of different size and position among patients. We are reporting a Mexican patient with typical features of KSS containing a novel deletion of 7437 bp in size not reported previously with 85% of heteroplasmy. Sequence analysis revealed two imperfect short repeats flanking the deletion regions as follow: AGAACCC/AGAAACC and ATACAT/ATCAT. Besides, sequencing, alignment and phylogenetic analysis of the hypervariable region revealed that the patient belongs to the Native American haplogroup C.

P04-30
Alginate/chitosan nanoparticles as tamoxifen controlled delivery formulations. Synthesis and characterization studies

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Nanomedicine attempts to use sophisticated approaches to either kill specific cells or repair them one cell at a time, offering new possibilities towards the development of personalized medicine focused on certain diseases which are currently being investigated, especially cancer. The first generation of nanoparticles comprises passive delivery systems that, in case of cancer, reaches the tumour through the fenestrations in the adjacent neovasculature. The unique mechanism of driving systems to the tumour site is the size of particles, not specific recognition of the tumour or neovascular targets. Nanoparticles based on mixtures of alginate and chitosan have been synthesized by an emulsification method and stabilized by amide bond formation between both polymers. These nanoparticles were assayed as drug delivery systems by loading them with tamoxifen (TMX). Results showed the formation of spherical nanoparticles with very small size (19–28 nm). The amide bond formation between both reactants was determined by FT-IR and confirmed by TGA studies, which also showed the thermal stability of both formulations. TMX was successfully incorporated into the systems (2–3 μg TMX/mg NP). Maximum TMX release took place between 8 and 24 hour, and interaction between TMX and the

system was dependent on nanoparticle composition, being the composition with higher proportion of alginate the one which showed the best release control.

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P04r-31
DNA-PK inhibitors NU7026 and NU7441 as enhancers of topoisomerase II poisons cytotoxicity

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DNA-dependent protein kinase (DNA-PK) is a DNA-activated serine/threonine protein kinase, abundantly expressed in almost all mammalian cells. DNA-PK plays a critical role in DNA-damage repair pathways, especially in non-homologous end-joining (NHEJ) repair system. DNA DSBs are considered the most cytotoxic type of DNA lesion. DNA DSBs result from endogenous events such as the production of reactive oxygen species, as well as from exogenous sources such as ionizing radiation and topoisomerase II (TOP2) poisons. When DNA is unrepaired, these lesions can result in cell death. Inhibition of NHEJ repair system is therefore an attractive approach to modulate resistance to therapeutically induced DNA DSBs. NU7026 and NU7441 are both potent, specific DNA-PK inhibitors investigated in models of human cancer. We measured chemosensitization by NU7026 and NU7441 of topoisomerase II poisons such as mitoxantrone and antimitotic drugs such as vincristine and taxol on cells from prostatic tumor origen PC3. Flow cytometry methods and cell cycle markers were used to assess the effect of mitoxantrone and antimitotic drugs on cell cycle. Cytotoxicity assays were carried out to measure chemosensitization by NU7026 and NU7441 to those drugs. Both inhibitors increased the cytotoxicity of mitoxantrone, and NU7026 potentiated vincristine and taxol effects. Alkaline comet assays were used to assess the induction of DNA DSBs. Mitoxantrone produced DNA DSB formation. Combining mitoxantrone with DNA-PK inhibitors, enhanced DSBs are observed at low doses (up to 0.2 μM) of TOPII inhibitors while at high doses (up to 2 μM) of TOPII inhibitors the effect is more complex. Finally, DNA damage was repaired after washing out mitoxantrone from cells and putting them on fresh media. NU7441 prevents partially DNA repair after washing out mitoxantrone while this reversion is not so evident with NU7026.

P04-32
Clinical significance of serum soluble CD163 levels in patients with hepatocellular carcinoma

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Aim: Soluble CD163 (sCD163) has been identified as a macrophage/monocyte-specific plasma protein and increased concentrations have been measured in patients with liver disease of different stages and severity. The aim of the present study was to evaluate the correlation between serum soluble CD163 (sCD163) levels and clinicopathological features in patients with hepatocellular carcinoma (HCC).

Methods: Eighteen patients with potentially resectable hepatocellular carcinoma were recruited in the study. Preoperative serum levels of sCD163 were measured by Macro 163^{TM} diagnostic kit.

Results: Serum sCD163 level in HCC patients was inversely correlated with serum albumin level ($p < 0.01$), and positively correlated with serum bilirubin level ($p < 0.001$). Serum sCD163 level was not associated with tumor characteristics such as tumor size, presence of microsatellite nodules, tumor grade and tumor stage. Serum sCD163 level was significantly higher in HCC patients with cirrhosis compared with those without cirrhosis ($p < 0.001$). Furthermore, a significantly better disease-free survival was observed in HCC patients with low sCD163 level ($p = 0.023$).

Conclusions: Serum soluble CD163 level appears to reflect the severity of underlying chronic liver disease rather than the tumor status in HCC patients, and low preoperative serum sCD163 level is predictive of better disease-free survival after surgery.

P04-33
Haemoglobin polymerization inhibition potentials of crude aqueous and ethanolic extracts of Pleurotus squarossulus and Pleurotus tuber-regium

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Edible foods derived-nutraceuticals may deter aggregation of deoxygenated sickle cell hemoglobin (deoxyHbS) molecules. This *in vitro* study evaluated the capacity of two edible mushrooms, *Pleurotus squarossulus* and *Pleurotus tuber-regium* to alter polymerization of HbS. The level of polymerization of hemolysate HbS molecules treated with sodium metabisulfite at 2 min intervals over period of 30 min in the presence of aqueous and ethanolic extracts of *P. squarossulus* and *P.tuber-regium* was monitored by spectrophotometry. *In vitro* effects of the crude aqueous extracts (CAE) and crude ethanolic extracts (CEE) of *P. squarossolus* and *P. tuber regium* on Fe^{2+}/Fe^{3+} ratio relative to phenylalanine equivalent was also investigated. CAE and CEE of *P. squarossolus* exhibited the highest capacity to deter polymerization of deoxyHbS molecules *in vitro* [97.5 ± 0.5% (CAE at 40 mg/ml) and 96.7 ± 1.2% (CEE at I20 mg/ml)]. The highest relative % polymerization inhibition obtained for CAE of *P. tuber-regium* (90.0 ± 8.4) was at 120 mg/ml while its CEE gave 90.0 ± 8.0% at same concentration. MetHb% in the HbSS blood in the presence of phenylalanine was 15.4 ± 0.0% and Fe^{2+}/Fe^{3+} was 5.6 ± 0.0%, in the presence of the extracts of *P. squarossolus* (CEE and CAE) Met HB% was 4.9 ± 0.4 and 7.5 ± 0.5%, while Fe^{2+}/Fe^{3+} was 19.7 ± 2.0% and 12.4 ± 0.1% respectively. In the presence of CEE & CAE extracts of *P. tuber-regium,* Met Hb% was 20.1 ± 5.9% and 5.0 ± 0.1% while Fe^{2+}/Fe^{3+} ratio was 4.3 ± 1.7% and 19.2 ± 0.7% respectively. The findings strongly suggest that extracts from *P. squarossulus* and *P. tuber-regium* could be useful adjuvant in the management of sickle cell anaemia.

Key words: haemoglobin polymerization; sickle cell anaemia; *Pleurotus squarossolus*; *Pleurotus tuber-regium, sclerotium*

P04-34
Expression, function and targeting of calcitonin receptor in glioma cells of the brain tumour glioblastoma multiforme (GBM)

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With a mean survival from diagnosis of 14 months. The calcitonin receptor (CTR) is best characterised for its role in bone/calcium homeostasis but is also implicated in cardiovascular disease, wound healing and some cancers. In this study, the expression of CTR was investigated using antibodies against two separate CTR epitopes in biopsies of brain tumours from patients diagnosed with GBM. CTR expression was associated predominantly with cells that resembled malignant glial cells and brain tumour initiating cells (BTIC, positive for GFAP, nestin and CD133), and was largely absent from surrounding cells and structures. CTR + ve cells were found in 12/14 GBM tumours ($p < 0.05$). In a transformed glioblastoma cell line (A172), calcitonin stimulated adenylyl cyclase activity and inhibited phosphorylation of ERK1/2. To understand the function and exploit the expression of CTR in tumour cells, high-grade glioma (HGG) cell lines (equivalent to BTICs) were investigated. We demonstrated expression of CTR in HGG lines and investigated ERK1/2 phosphorylation and cAMP generation in response to calcitonin. To investigate CTR as a therapeutic target, we utilised an antagonist form of calcitonin and an extracellular domain-directed antibody against the CTR. The uptake of fluorophore-conjugated forms of the antagonist and antibody in CTR-transfected Cos7 and endogenously expressing HGG cell lines was tested, as well as their effect on HGG cell survival. The expression of CTR in GBM biopsies and HGG cell lines, along with functional characterisation in HGG cell lines, indicate that CTR might be a useful target in GBM.

P04-35
Global and gene specific methylation profile in Sardinian patients with Acute Myocardial Infarction

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Acute myocardial infarction (AMI) is a clinical state induced by the thrombus formation following the disruption of unstable atherosclerotic plaque. Atherosclerosis is known to be an inflammatory process mediated by several risk factors, such as prostaglandins synthesized by the enzyme cyclooxygenase (COX). In this study, we examined the global and gene specific methylation profile to evaluate its involvement in AMI Sardinian population. The study included 118 subjects: 51 healthy controls and 67 AMI patients. Genomic DNA was extracted from peripheral blood leukocytes using the Salting Out method. Sodium bisulfite conversion was conducted on target sequence. Global DNA methylation was determined by Capillary Electrophoresis. COX2 promoter methylation was analyzed by MSP. The percentage mean of methylated to total cytosine was higher in AMI patients than in healthy controls; this difference was statistically significative (4.004 ± 0.525 versus 3.835 ± 0.362; $p = 0.043$). Moreover, it has been observed a positive interaction of gender on percentage of methylated to total cytosine; particularly, male AMI patients showed significant higher value of global DNA methylation. An incomplete COX2 promoter methylation has been find in both groups; all samples showed the presence of both methylated and unmethylated bands. No significative difference has been observed between AMI and control patients about COX2 methylation frequencies. There is no evidence of an interaction effect of gender on COX2 methylation frequencies. These preliminary results indicate that global DNA methylation may play an important role in AMI process in Sardinian population. Further studies are required to elucidate the involvement of gene specific methylation in atherosclerotic and thrombotic pathways.

P04-36
Cardiovascular effects of a novel selective Rho kinase inhibitor, 2-(1H-indazole-5-yl)amino-4-methoxy -6-piperazino triazine (DW1865)

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The arising critical implications of Rho-kinase (ROCK) signaling in cardiovascular diseases have growing interest in the pharmacological potential of ROCK inhibitors. We identified a novel inhibitor of ROCK (DW 1865; 2-(1H-indazole-5-yl)amino-4-methoxy -6-piperazino triazine) and characterized its effects in biochemical, cellular, tissue and animal-based assays. DW 1865 potently inhibited the kinase activity of both ROCK1 and ROCK2 *in vitro*, and behaves as an ATP-competitive inhibitor. Interestingly, DW1865 was 10 times more effective on inhibiting ROCK kinase activities than Fasudil, a ROCK selective reference compound. Its activity is highly selective in panel assay of 13 other kinase. In isolated vascular tissue study, DW1865 as a vasorelaxant drug was ≥3 times more effective than fasudil. In SHR, acute administration of DW1865 caused significant and dose-related reduction in blood pressure. Moreover, DW1865 blocked angiotensin II-induced stress fiber formation and cellular hypertrophy in rat heart-derived H9c2 cell. In conclusion, DW1865 possess beneficial effects of ROCK inhibition go beyond blood pressure lowering to include potential reduction of hypertrophy, fibrosis in cardiomyocytes. DW1865 may be useful for pharmaceutical or clinical applications as an effective ROCK inhibitor.

P04-37
Recombinant glycoprotein from yeast for immunodiagnostic of HSV-2

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Serodiagnosis of herpes simplex virus type 2 (HSV-2) infections have been difficult to carry out because antibodies to HSV-1 show an extensive cross-reactivity with HSV-2 antigens. The yeast expression of recombinant proteins is an important tool to obtain post-translational modifications, similar to the mammalian expression, which can be essential in its activity and/or antigenicity. Attempts to develop recombinant antigens to allow discrimination of exposure to alphaherpes viruses by ELISA have been conducted without obtaining enough sensitivity and specificity. Due to this fact, a recombinant glycoprotein G (gG2) from HSV-2 has been expressed in *Saccharomyces cerevisiae*, using 6× histidine and GST as reporter gene to get the fusion protein. Two strains with different genetic background were selected and studied both in selective and in rich medium, maintaining a plasmid stability of more than 99% in the absence of selective pressure. The improvement of the

expression conditions in a bioreactor were carry out with the control of parameters such as pH, time, OD, temperature, dO_2 and agitation, in order to reduce degradation and to obtain higher biomass. Moreover, with the addition to the medium of additives which induce the glycosylation, the ratio of glycosylated gG2, has been improved. After yeast cell disruption and clarification, the purification strategy by affinity chromatography using the tag followed by lectin-agarose, allow to obtain a rich glycosylated antigen to use in immunodiagnostic of HSV-2 with similar results, regarding antigenicity, to the native antigen. Advantages include high yield, high productivity, and significant cost and time savings over insect and mammalian expression systems.

P04-38
System biology approaches for the interrogation of anti-malarial compounds

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Systems biology holds promise as a new approach to drug target identification and drug discovery against many tropical diseases. Model based techniques have yielded valuable insight into critical processes involved in the interaction between the parasite and the host (1). Analysis of the host-pathogen interaction can be used to predict critical protein targets that when perturbed singly or in combination have adverse effects on virulence and/or growth. Here, a model of key processes involved in the erythrocyte infection and parasite gametogenesis is proposed that generates a set of high-priority host and *P. falciparum* targets, which can be eventually associated with some temptative or already approved drugs that are antimalarial candidates. Candidate drugs can begin to be investigated for clinical use against malaria. In addition, the selection of high-priority double-drug combinations that demonstrate superadditivity might provide for an attractive and alternative avenue for drug discovery against malaria. The interrogation of publicly available resources allows us to present significant results to future drug discovery and drug repurposing strategies against malaria.

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P04-39
The effects of folk medicinal plant viscum album I. On protein and mRNA expressions of CYP27A1 in rat liver

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Viscum album is a common bushy plant of the family Viscaceae, which grows as epiphyte on the branches of deciduous trees. It is a medicinal plant widely distributed in tropical and subtropical Africa, in Asia and in Europe. The plant contains a great variety of compounds including lectins, viscotoxin, polysaccharides, flavonoids, phenylpropane derivates, triterpenoids and phytosterols. The use of *Viscum album* in the treatment of breast, lung and liver cancers and cardiovascular diseases indicates pharmaceutical importance of this plant. In mammals, excess cholesterol is removed mainly through conversion to bile acids. Cytochromes P450s initiate all quantitatively significant pathways of cholesterol metabolism and bile acid biosynthesis. There are two pathways namely the classical pathway and the acidic pathway involved in bile acid synthesis. Sterol 27-hydroxylase (CYP27A1) is a mitochondrial CYP450 enzyme found most cells and catalyzes both initial and rate limiting step in the acidic pathway. In this study, the possible potency of medicinal plant *Viscum album* L.extracts on rat liver CYP27A1 was investigated. The water extract of *Viscum album* was injected into rats intraperitoneally as 10 mg/kg for 9 days. *In vivo* effects of *Viscum album* L. on rat liver were analyzed by determining protein expression level using western blotting technique and mRNA expression by using qRT-PCR. The results have shown that injection of viscum album extract decreased the CYP27A1 protein level 24% with respect to controls ($p < 0.005$). mRNA expression studies also confirm this change. In conclusion, metabolism of bile acids by CYP27A1 catalyzed reactions may be altered due to the changes in mRNA and protein expressions of this enzyme by *Viscum album* L. extract.

Keywords: *Viscum album* L., bile acids, CYP27A1, mRNA and protein expression, rat liver

P04-40
The effect of Miglustat on the enzymatic function of intestinal disaccharidases

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Miglustat is an oral medication that has approved indication for treatment of type I Gaucher disease and has been tested for some other glycolipid storage diseases. This compound is an N-alkylated imino sugar which functions through substrate reduction therapy by inhibiting the primary step of sphingolipid glycosylation in the Golgi. The immediate side effects of this drug target carbohydrate digestion in the intestinal tract, leading to occurrence of gastrointestinal intolerances with symptoms similar to congenital disaccharidase disorders. These observations highly suggest that the function of the intestinal disaccharidases has been affected. In this study, we examined the direct effect of Miglustat on the enzymatic activity of the major intestinal disaccharidases such as sucrase-isomaltase, maltase-glucoamylase and lactase-phlorizin hydrolase from human intestinal brush border membrane. Our data show that the α-glucosidic activity of the intestinal disaccharidases is substantially inhibited by Miglustat, while lactase as a β-galactosidase is only affected at high non-physiological concentrations. Further analysis of the kinetics of inhibition revealed that as a non-cleavable substrate, Miglustat can strongly compete with sucrase, isomaltase and maltase at their relative active sites, in addition to a less-influential uncompetitive inhibitory function. As a result, maldigested carbohydrates are accumulated in the intestinal lumen which leads to appearance of osmotic diarrhea and other gastrointestinal intolerances. This study has been supported by Actelion Pharmaceutical GmbH.

P04-41
The antimicrobial activity of fabricated iron oxide nanoparticles

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Iron oxide magnetic nanoparticles have been widely used in a variety of biomedical applications such as magnetic separation, magnetic resonance imaging, hyperthermia, magnetically-guided drug delivery, tissue repair, and molecular diagnostics. The antimicrobial activities of the newly synthesized compounds were determined against microbial clinical and ATCC reference strains, i.e., *Bacillus subtilis* and *Escherichia coli* ATCC 25922. The aim of this study was to evaluate the toxic effect of iron oxide (IO) nanoparticles (NPs) on Bacillus subtilis and *E. coli* ATCC 25922. The microbial strains identification was confirmed using the VITEK II automatic system. The VITEK cards for identification and susceptibility testing (GNS-522) were inoculated and incubated according to the manufacturer's recommendations. The results were interpreted using the software version AMS R09.1. The inoculated plates were incubated for 24 hrs at 37°C. Antimicrobial activity was assessed by measuring the diameters of the growth inhibition zones expressed in mm. In our study the IO-NPs proved to inhibit the growth of microbial cells, as demonstrated by the absorbance measurements at 620 nm of the obtained cultures. The antimicrobial activity of fabricated IO-NPs was examined using two common bacterial pathogens, *E. coli* (gram negative) and *Bacillus subtilis* (gram positive). Increasing concentrations of IO-NPs (0.01 to a 5.0 mg/ml) inhibited the growth of *E. coli* and *Bacillus subtilis* progressively in a dose-dependent manner. *E. coli* bacterial growth was completely inhibited at the highest concentration of IO-NPs (1.25 mg/ml). Bacillus subtilis was more sensitive than *E. coli* to the NPs and no growth was observed at a concentration of 0.156 mg/ml. An interesting result was the different behaviour of the two strains when they were exposed to the same concentration of IO-NPs. Bacillus subtilis was more sensitive to the addition of NPs than *E. coli* ATCC 25922. We were also able to demonstrate that the antimicrobial activity was dependent on the size of the NPs.

P04-42
Evaluation of antibacterial effect of silver doped hydroxyapatite nanoparticles

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HAp, $Ca_{10}(PO_4)_6(OH)_2$, is being used in medical implants for the repair and reconstruction of diseased or damaged hard tissue due to the good biocompatibility and osteoconductivity. One of the most common problems with using implants is the risk of developing post operatory infections or having the implant rejected by the body. HAp, $Ca_{10}(PO_4)_6(OH)_2$, is the ideal biomaterial to embed silver ions in because the Ca^{2+} ions can be easily substituted by Ag^{+} ions in the matrices, creating a material with high biocompatibility and antibacterial properties. This study showed that silver doped hydroxyapatite nanoparticles (Ag:HAp-NPs) with $0 \leq x_{Ag} \leq 0.2$ presented a good antibacterial activity against Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 25922). The antimicrobial activity of Ag:Hap-NPs was tested using the standard microdilution method. Staphylococcus aureus (ATCC 6538) and Escherichia coli (ATCC 25922) were grown on LB agar broth with five different concentrations of Ag:HAp-NPs (5, 10, 50, 75, 100 μg/ml) with $0 \leq x_{Ag} \leq 0.2$. The viable organisms from the buffer were quantified by plating serial dilutions on yeast extract agar plates. Yeast extract agar plates were incubated for 24 hour at 37°C and the obtained colony forming units (CFU) were visually counted.The Ag:HAp-NPs show strong antibacterial activity. The *in vitro* bacterial adhesion study indicated a significantly reduced number of *E. coli* and *S. aureus* on different concentration of Ag:HAp-NPs ($0.1 \leq x_{Ag} \leq 0.2$). For the samples Ag: HAp with $x_{Ag} = 0$ the inhibitory activity is not present. The results of this study clearly demonstrated that the Ag:HAp-NPs with $x_{Ag} \geq 0.1$ inhibited the growth and multiplication of the tested gram-positive and gram-negative bacteria, including highly multidrug-resistant bacteria such as *S. aureus* and *E. coli*. The development of novel nanoparticles covering the surfaces of ambulatory and other medical devices would provide an alternative means to decrease the microorganism colonization and device-associated infection.

P04-43
Aureobasidium culture supernatant synergistically stimulated R-848 activated phagocytosis of PMA-induced THP-1 macrophages

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Toll-like receptors (TLRs) have an important role in innate immunology, which recognize a wide range of microbial pathogens and pathogen-related products. Macrophages have variety of TLRs and pathogen binding to TLR resulted in activation of macrophages. R-848, immune response modifier, is an analog of imidazoquinoline and binds to endosome-localized TLR, TLR7 to exert anti-viral response. In the present study, we verified that co-treatment of R-848 and other TLR agonist would enhance immune response. In this study, we used an *in vitro* macrophage model. As human monocyte leukemia THP-1 cells were treated with PMA, cells developed a macrophage-like type cells. We used PMA-THP-1 macrophages throughout this study. R-848 and aureobasidium culture supernatant (contains β-glucan mainly), which binds to other cell membrane localized TLRs, TLR-2 or TLR-4, and also to Dectin-1, was treated to THP-1 macrophages. Co-treatment of R-848 and aureobasidium culture supernatant significantly augmented various cytokines expression such as TNF-α and IL-12, compared to the treatment alone. Next, we investigated apoptotic cell uptake would be altered. For detecting apoptotic cells, we induced apoptosis to human lymphoma Jurkat cells by 5-FU and stained with TAMRA for detecting apoptotic cell uptake. Co-treatment of R-848 and aureobasidium culture supernatant significantly augmented phagocytosis of apoptotic Jurkat cells. These results suggest thatactivating several different TLRs may enhance immune response synergistically.

P04r-44
When sperm gets drunk: lifestyle factors influence sperm quality

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The high incidence of low sperm counts in young European men and the decline in sperm counts in recent decades suggests that

environmental and/or lifestyle factors play an important role in male fertility. In fact, lifestyle factors, namely alcohol consumption, cigarette smoking and use of illicit drugs have been shown to induce adverse effects on male reproduction. In Portugal there is a strong tradition of alcohol, tobacco and drug abuse during academic week festivities, thus offering exceptional natural conditions for studying these abusive factors in a young and healthy population over a fixed and acute period of time. Our study aimed at searching a relationship between sperm quality, an apoptotic marker and abuses that occur during academic week festivities. Conventional semen parameters and expression of cleaved Poly ADP-ribose polymerase (PARP) protein, an apoptotic marker, were analyzed on 54 and 35 young male volunteers, respectively, before and after the Portuguese academic week festivities, in 2010. Our results demonstrated that acute life style alterations during academic week festivities were associated with a significant increase of 53% in cleaved PARP in ejaculated spermatozoa ($p = 0.0003$), a concomitant decrease in sperm concentration ($p = 0.007$), in the total number of spermatozoa in the ejaculate ($p = 0.0008$), in volume ($p = 0.00182$) and increased sperm morphological defects ($p = 0.00076$). The acute life style alterations have clear deleterious effects on sperm quality. Hence we propose cleaved PARP as a novel molecular biomarker, valuable for assessing sperm quality.

P04r-45
Understanding how tumor microenvironment governs breast cancer plasticity and malignancy *in vitro*

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Breast tumors are classified based on molecular and hormone receptor status. Two of these categories include triple negative (TNBC, ER-/PR-/HER2-) and luminal breast cancer (LBC, HER2 + /PR + /ER +). Breast cancer recurrence rates are variable, suggesting that tumor cells disseminate from primary sites at an early stage but remain indolent for extended periods of time before progressing to symptomatic disease. Little is known about mechanisms that cause otherwise indolent tumors to become overt cancers, making it difficult to predict which breast cancer patients are likely to relapse and so, to benefit from preventive therapy. Elements of the tumor microenvironment are crucial regulators of cancer cell growth and homeostasis, and these factors certainly influence the course of tumor progression. However, the mechanisms by which indolent tumor cells acquire malignant properties in response to tumor-supportive systemic and local environments are not clear. We previously reported that certain human carcinomas ('Instigators') facilitate the growth of indolent tumor cells ('Responders') located at distant anatomical sites, through the mobilization of bone marrow cells that create a permissive pro-tumorigenic microenvironment, in a process called 'Systemic Instigation'. In preliminary experiments we have: (i) Generated an *in vitro* model that approximates *in vivo* instigation processes and (ii) Determined the tumorigenic ability, gene expression, and phenotypic plasticity of responder tumor populations after TNBC or LBC systemic instigation. Our preliminary results suggest that TNBC and LBC tumors govern the composition of the responder tumor microenvironment and they influence the differentiation status of responding tumors. Specifically, percentages of progenitor cells (CD44 + /CD24–) and differentiated cells (CD44 + /CD24 +) change depending on the microenvironment. These experiments set the stage for our long-term goal which is to fully characterize this *in vitro* system and to translate our findings to circulating tumor cells isolated from peripheral blood of breast cancer patients.

P04-46
Impact of chronic exposure to heavy metals and tobacco smoke on the formation of advanced oxidation protein products

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Advanced oxidation protein products (AOPP) are compounds formed by influence of oxygen free radicals, especially chloramine oxidants generated by myeloperoxidase activity on the native structure of proteins. AOPP are the most often derivatives of albumin and fibrinogen, but also probably lipoproteins, glycoproteins and immunoglobulins, resulting from the aggregation of protein molecules and the formation of cross-linkages. The aim of study was to determine the relationship between exposure to heavy metals and tobacco smoke on the concentration of AOPP in the plasma of steelworkers. Level of AOPP was determined in the plasma of 84 steelworkers exposed to heavy metals (40 smokers, 44 non-smokers) and 51 control subjects, not exposed occupationally to heavy metals (13 smokers and 38 non-smokers). AOPP concentration was determined by spectrophotometric method developed by Witko-Sarsat *et al.* (1996). Cotinine concentration was determined by ELISA. It has been shown statistically significant difference between the concentration of AOPP in the plasma of smoking steelworkers (66.99 ± 22.91 μmol/l) and the level of AOPP in the plasma of control subjects, both smokers (30.85 ± 12.95 μmol/l) and non-smokers (25.93 ± 10.31 μmol/l). A difference between the concentration of AOPP in the plasma of non-smoking steelworkers (70.12 ± 23.31 μmol/l) and levels of AOPP in the plasma of control subjects, both smokers and non-smokers, was demonstrated. Differences were not observed between the concentration of AOPP in the plasma of smoking and non-smoking steelworkers and between smoking and non-smoking control subjects. Environmental exposure to heavy metals results in imbalance in pro/antioxidant status and increases the concentration of AOPP.

P04-47
Synergistic anti-tumor effects of radiation and beclin1 via regulating autophagic pathway in the lungs of K-ras^{LA1} mice

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Radiotherapy alone has several limitations in treating lung cancer. Inhalation, a non-invasive approach for direct delivery of therapeutic agents to the lung, may help to enhance therapeutic efficacy of radiotherapy. Up-regulating beclin1, known as a tumor suppressor gene which plays a major role in autophagy, via inhalation may sensitize tumors and lead to tumor regression in lungs of K-ras^{LA1} lung cancer model mice. To minimize the side effect of radiotherapy, fractionated exposures (five times, 24 hour interval) with low dose (2Gy) of radiation to restricted area (thorax, 2 cm) were attempted. After sensitizing the lungs with radiation, beclin1, complexed with a nano-sized biodegradable poly(ester amine), was prepared and delivered into the mur-

ine lung via aerosol three times/week for 4 weeks. Animals treated with beclin1 and radiation showed highly significant tumor regression, with low progression to adenocarcinoma in histopathological analysis. Increment in number of autophagic vacuoles and secondary lysosomes was detected. Dissociation of beclin1-bcl2 stimulated autophagy activation and showed synergistic anti-tumor effect through inhibition of Akt-mTOR pathway, cell proliferation and angiogenesis. Combination of radiation with non-invasive aerosol delivery of beclin1 may provide a prospect for developing novel therapy regimens applicable in clinics.

P04-48
Effects of real-time multiplex SYBR Green I for simultaneous detection of *Brucella* and *M. tuberculosis* complex

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SYBR Green I (SGI) is a double-strand-specific intercalating DNA dye widely used in real-time PCR applications that allows monitoring of product formation and melting temperature (Tm) of one or more amplicons. It is also cheaper than other detection formats. When using SGI in real-time multiplex PCR, discrimination of amplicons is possible if the Tm values differ sufficiently. The Tm, therefore, can be used for clinical diagnosis under defined conditions of assay reproducibility. This strategy is especially useful in situations where various microorganisms cause similar clinical syndromes. Our aim was the development and evaluation of nine real-time multiplex PCR for the simultaneous detection of *Brucella* spp and *Mycobacterium tuberculosis* complex (MTC). We chose three targets with different numbers of copies in the genome for each microorganism. The IS711, bcsp31 and Omp2a genes were used for the identification of *Brucella* spp and the IS6110, senX3-regX3 and cfp31 genes were targeted for the detection of members of the MTC. Tm analysis showed different simultaneous detection efficiencies in multiplex assays, related to the GC% content of the amplicons. In contrast, the analysis of the products on agarose gels revealed that both gene sequences were being amplified in all nine gene combinations. The fluorescence emitted in the reactions was lower in multiplex with multicopy elements, showing the saturation effect exerted by the SGI in the reaction. In conclusion, the GC% content of amplicons and the presence of multicopy elements hinders or impedes the simultaneous detection in multiplex reactions, which must be considered when designing SGI assays.

[Correction after online publication 30 August 2012: Authors and presenter names, ''R. Sanjuán-Jiménez, P. Bermudez and J. D. Colmenero'' were corrected.]

P04-49
Pentamethine salts for superior fluorescence imaging of mitochondria

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Labeling of mitochondria for fluorescence microscopy is generally achieved using transiently expressed mitochondrial protein markers or dyes specifically accumulating in this organelle. We demonstrate a series of novel fluorescent dyes from γ-aryl substituted pentamethine family possessing excellent photostability, fluorescence properties and low phototoxicity. They localize in mitochondria of various cell lines with unique selectivity and are detectable in nanomolar concentrations. Our results indicate that these novel mitochondrial dyes effectively cross the cell plasma membrane and then accumulate in inner mitochondrial membrane due to binding to cardiolipin. Pentamethine salts label mitochondria with high specificity and their low toxicity enables to study morphological changes and structural complexity of these dynamic organelles in different cell lines in real time by live cell fluorescence microscopy. Moreover, they are suitable also for mitochondria staining in fixed cells as they are retained during washing and fixation procedures.

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P04-50
Biscarbamate derivatives of bronchodilators are potent and selective butyrylcholinesterase inhibitors

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A very desirable characteristic for an asthma drug is prolonged action, providing a patient with a whole night's sleep. This request is met by bambuterol, a biscarbamate prodrug of terbutaline, whose high therapeutic index of bambuterol is associated with its extremely selective inhibition of butyrylcholinesterase (BChE) compared to acetylcholinesterase (AChE). Metacarb and isocarb, newly synthesised biscarbamates of bronchodilators metaproterenol and isoproterenol, are structurally similar to bambuterol and we expected that they should have similar inhibition potency and selectivity in inhibition of BChE. Metacarb and isocarb proved to be very potent BChE inhibitors with 2.2 and $0.2 \cdot 10^6$ M/min inhibition rate constants, and very selective BChE inhibitors, as they inhibited AChE 960 to 80 times more slowly than BChE, respectively. To elucidate the inhibition potency of studied biscarbamates and bambuterol, we used molecular modelling to study the transition state of carbamylation reaction. Differences in carbamylation rate by metacarb, isocarb and bambuterol can be explained by additional stabilization typical for each carbamate: metacarb by two hydrogen bonds with residues His438 and Glu197, isocarb by the hydrogen bond with Glu197, and bambuterol by the cation–π interaction between protonated nitrogen and Tyr440, and by the hydrogen bond with Glu197. In conclusion, metacarb and isocarb proved far less selective for BChE than bambuterol, and therefore less likely to be used as prodrugs of bronchodilating agents.

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P04-51
Elaboration of sensitive immuno-PCR techniques for diagnostics of bacterial infections

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Development of fast and sensitive diagnostic tools for the detection of bacterial pathogens forms a cornerstone of successful antibacterial therapy and prevention of disease outbreaks. Being one of the most sensitive among modern detection techniques, immuno-PCR appears to be tempting strategy for utilization in early detection of life-threatening infections. Pursuing the goal to choose an efficient strategy for the construction of immuno-PCR diagnostic kits detecting various bacterial pathogens, we have explored a spectrum of approaches for the construction of antibody-DNA conjugates and analyzed the benefits of different immuno-PCR strategies. Applying pairs of monoclonal antibodies raised against different epitopes of the target protein, we have developed diagnostics kits for highly specific detection of *B. anthracis* lethal toxin components: lethal factor and protective antigen. Detection limits for these antigens were as low as 10^{-12} g/ml. A trial approach aimed at the development of immuno-PCR based on chemical conjugates of DNA with rabbit polyclonal antibodies specific to bacterial surface or to individual protein markers, turned out to be promising strategy for rapid development of diagnostic tools for the detection nosocomial infections caused by *A. baumannii,* NDM-1-carrying bacterial strains, and hazardous gastrointestinal disease, caused by enteropathogenic *E. coli* exemplified by the O157:H7 strain. The results obtained indicate that chemical conjugates of antibodies to DNA displayed superior performance comparing streptavidin-biotin counterparts thus representing promising approach for the development of immuno-PCR diagnostic tools.

P04-52
Significance of NSE in various cancer types

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Neuron specific enolase (NSE) is one of the three enolase isoenzymes found in mature neurons and cells of neuronal origin. It is detected in patients with tumors of neuroendocrine origin and small cell lung cancer (SCLC). Measurement of NSE levels in patients with these diseases can provide information about the patients' prognosis and the response to treatment. The aim of the study is to investigate the serum concentrations of NSE in patients with cancer and compare with the healthy controls to clarify the role of NSE in progression of various cancer types. Serum samples taken from 184 patients (76 prostate cancer, 34 Hodgkin lymphoma, 19 Non-Hodgkin lymphoma, 14 lung cancer, 22 peripherical nerves tumor, 19 brain tumor) and 132 healthy controls were analyzed for NSE concentration using an enzyme-linked immunosorbent assay (ELISA) in I.U.Oncology Institute, Cancer Biochemistry and Tumor Marker Laboratory. The results were evaluated by the Student-*t* test using SPSS 16 (Chicago, IL, USA). p values < 0.05 were considered as statistical significant. The mean serum NSE value observed in the healthy subject was 18.6 ± 4.7 ng/ml. According to the test, the mean serum NSE levels of prostate cancer ($p = 0.001$), Hodgkin lymphoma ($p = 0.01$), lung cancer ($p = 0.018$) and peripherical nerves tumor ($p = 0.008$) patients were significantly higher than the serum NSE levels of the healthy controls. Our data indicate that high serum levels of NSE can be used as a diagnostic and prognostic parameter for prostate cancer, Hodgkin lymphoma, lung cancer and peripherical nerves tumor.

P04-53
Isolation and characterization of the human acute phase protein ITIH4 (inter-α trypsin inhibitor heavy chain 4 protein)

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ITI family includes a group of plasmatic proteins that result from different combinations of several heavy chains (H1, H2, H3, H4 y H5) and a light chain. Previous studies from our laboratory focused on the characterization of the major acute phase protein in pigs (pigMAP) which is highly induced during the porcine acute response. This protein shows a high homology with human protein PK-120 and is considered a member of ITI family. In fact, human protein PK-120 is also known as ITIH4 since it contains the H4 chain of the family. Several studies highlighted the interest of ITIH4 as a pathological marker and methods that allow its quantification in sera are under development. To address this subject we isolated ITIH4 from blood donors using a combination of ion exchange, Sepharose 4B-inmobilized Cibacron Blue and molecular exclusion chromatography. A preparation of purified ITIH4 was used as an immunogen to raise antibodies in rabbits. Anti-ITIH4 antisera, after immunoadsorption, were suitable for the quantitative determination of the protein. ITIH4 concentration was determined in serum samples from patients with Systemic inflammatory response syndrome (SIRS) using our specific antibodies. Samples from healthy donors were used as control. An increase in the ITIH4 concentration was observed reaching values up to 0.7 mg/ml, approximately 2.5 times higher than those found in controls. In conclusion, the human positive acute phase protein ITIH4 exhibited moderate increases when compared with those observed for CRP, haptoglobin or $\alpha_1$acid-glycoprotein during acute phase processes.

P04-54
Leptin levels in breast cancer

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Breast cancer is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. While the overwhelming majority of cases are women, breast cancer also can develop sometimes in men. Treatment may include surgery, hormone therapy, chemotherapy and radiotherapy. Leptin is the product of the obese (*ob*) gene and is produced predominantly in white adipose tissue. Leptin, a protein produced mainly by adipose tissue, and may stimulate cancer growth, thereby mediating the effect of obesity on cancer risk. Some studies are reported that the increase of breast cancer risk depends on the high leptin levels. The aim of our study was to investigate the serum levels of leptin in breast cancer patients above 75 kg of weight.

Method: Our study group consisted of 78 breast cancer in I.U. Oncology Institute and 44 healthy controls. The serum levels of leptin parameters were measured by enzime-linked immunoassay (ELISA) in Cancer Biochemistry and Tumor Marker Laboratory.

Result: 78 cases of breast cancer were enrolled in the study. The mean (62.1), standart deviation (35.6), median (58.5) values in the breast cancer patients and the mean (0.89), standart deviation (0.37), median (0.98) values in the healthy controls were calculated by using SPSS software (SPSS 16, Chicago, IL, USA). Serum leptin ($p = 0.00$) levels were significantly higher in

patients with breast cancer than the healthy controls. Statistical significance was determined with the Mann–Whitney U test.
Discussion: Our data indicate that leptin can be used as a diagnostic parameter for breast cancer above 75 kg of weight. We believe leptin can be a useful marker for clinicians to help decide the diagnosis of breast cancer for obese women.

P04-55
Heme oxygenase-1 as a potential target for anticancer treatments in cholangiocarcinoma

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Cancer cells acquire drug resistance via various mechanisms including enhanced cellular cytoprotective and antioxidant activities. Heme oxygenase-1 (HO-1) is a key enzyme exerting potent cytoprotection, cell proliferation and drug resistance. Cholangiocarcinoma (CCA) is a malignant tumor of the bile duct, which originates from the bile duct epithelial cells. CCA is a devastating malignancy with poor prognosis.Many chemotherapeutic drugs have been tested as single agents or in combinations. Nevertheless, drug resistance or drug inefficacy remain major obstacles in the treatment of CCA.We aimed to investigate roles of HO-1 in human cholangiocarcinoma (CCA) cells for conferring cytoprotection against anticancer treatments including chemotherapeutic agents and radiotherapy. Inhibition of HO-1 by zinc protoporphyrin IX (ZnPP) sensitized both CCA cell types (KKU-100 and KKU-M214) to the cytotoxicity of chemotherapeutic agents; gemcitabine (Gem) and doxorubicin. HO-1 gene silencing by siRNA validated the cytoprotective effect of HO-1 on CCA cells against Gem. The concept has been assessed *in vivo* study. Mice bearing xenograft of CCA cells were treated with combination of Gem and HO-1 inhibitor. Treatment with gemcitabine and ZnPP significantly potentiates the effects of GEM to suppress tumor growth and reduced tumor weights. For radiotherapy, inhibition of HO-1 renders the CCA cells to be more sensitive to gamma radiation, leading to G2/M arrest and resulted in suppressed the proliferation. These results show that HO-1 played a critical role in cytoprotection in CCA cells against anticancer treatments. Targeted inhibition of HO-1 may be a strategy to overcome the resistance to anticancer treatments of bile duct cancer.

P04-56
Determination and significance of serum hepcidin and liver hepcidin mRNA levels in patients with hepatopathies

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Hepcidin is synthesized in the liver and plays a pivotal role in the regulation of iron metabolism by controlling both intestinal iron absorption and iron release from macrophages. Hepcidin synthesis is tightly controlled and responds to various pathological conditions. Chronic inflammation and iron overload up-regulate hepcidin synthesis in order to reduce plasma iron concentration. On the other hand, anemia and hypoxia down-regulate the production of hepcidin in order to increase iron availability. Evidence from experimental models suggests that infection with hepatitis C virus (HCV) also results to reduced synthesis of hepcidin, an effect that may contribute to liver iron accumulation and the progression of liver fibrosis to cirrhosis and hepatocellular carcinoma. To study the possible role of hepcidin in diverse liver disorders and determine its association with clinical patient characteristics, we measured the levels of serum hepcidin and liver hepcidin mRNA in over one hundred patients with HCV (n = 20) and HBV (n = 25) infections, primary biliary cirrhosis (PBC; n = 32), autoimmune hepatitis (n = 16) and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH; n = 15). Hepcidin mRNA levels were determined by extraction of total RNA from liver biopsy specimens and real-time quantitative RT-PCR. Hepcidin was quantified in patient sera drawn at the biopsy day using an immunological ELISA assay. We will present our results and their correlation with each specific liver disease as well as the demographic, laboratory, histological and clinical characteristics of patients and discuss their potential for disease development and diagnosis.

P04-57
Protein Phosphatase 1 complexes: targets for male infertility therapy

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Infertility is a growing concern in modern society, with 30% of cases being due to male factors, namely reduced sperm concentration, decreased motility and abnormal morphology. Sperm cells are highly compartmentalized, almost devoid of transcription and translation, consequently, processes such as protein phosphorylation provide a key general mechanism for regulating vital cellular functions, more so than for undifferentiated cells. Reversible protein phosphorylation is the principal mechanism regulating most physiological processes in eukaryotic cells. To date, hundreds of protein kinases have been identified, but significantly fewer phosphatases (PPs) are responsible for counteracting their action. This discrepancy can be explained in part by the mechanism used to control phosphatase activity, which is based on regulatory interacting proteins. This is particularly true for Phosphoprotein Phosphatase 1 (PPP1), a major serine/threonine-PPP, for which more than 200 interactors (PPP1 interacting proteins – PIPs) have been identified that control its activity, subcellular location and substrate specificity. For PPP1, several isoforms have been described, among them PPP1CC2, a testis/sperm-enriched PPP1 isoform. Recent findings support our hypothesis that PPP1CC2 is involved in the regulation of sperm motility. We focus our research on sperm-specific PP1-PIPs, involved in the acquisition of mammalian sperm motility and the potential relevance of targeting PP1-PIPs complexes to infertility diagnostics and therapeutics as well as to male contraception.

P04-58
Balance of human choline kinase isoform expression is critical for cell cycle regulation: Implications for the development of choline kinase-targeted cancer therapy

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The enzyme choline kinase (CK), which uses ATP for phosphorylation of choline to form phosphocholine (PCho), has an essential role in the biosynthesis of phosphatidylcholine, the major constituent of all mammalian cell membranes. CK is encoded by two separate genes expressing the three isoforms called CKalpha1, CKalpha2, CKbeta that are active as homo- or hetero-dimeric species. Metabolic changes observed in various cancer cell lines and tumors have been associated with differential and marked up-regulation of CKalpha genes, and specific inhibition of CKalpha activity has been proposed as a potential anticancer strategy. As a result, less attention has been given to CKbeta and its interaction with CKalpha. With the aim of profiling the intracellular roles of CKalpha and CKbeta, we used RNA interference as a molecular approach to down-regulate CK expression in HeLa and MCF-7 cells. Individual and simultaneous RNAi-based silencing of the alpha and beta isoforms was achieved using different combinations of knockdown strategies. Efficient knockdown was confirmed by immunodetection using isoform-specific antibodies, and by quantitative real-time PCR. Our analyses of the phenotypic consequences of choline kinase depletion showed the expected lethal effect of CKalpha knockdown. However, CKbeta- and double-silenced cells had no aberrant phenotype. Thus, our results support the hypothesis that the balance of the alpha and beta isoforms is critical for cancer cell survival. The suppression of the cancer cell killing effect of CKalpha silencing by simultaneous knockdown of both isoforms implicates that a more effective choline kinase-based anti-cancer strategy can be achieved by reducing cross-reactivity with CKbeta.

P04-59
Activity of gelatinases in chosen cancer cell lines

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Gelatinases are members of matrix metalloproteinase family – group of zinc/calcium-dependent proteolytic enzymes with capability of degradation of several matrix and non-matrix proteins. Because of intra- and extracellular activity and broad range of substrate specificity, gelatinases are considered to be an important factor in regulation of metastatic process, involved in gaining of cell motility, breakdown of extracellular matrix, and more. The role of gelatinases as putative diagnostic markers relies on the widely described differences in gelatinases' expression patterns in tumor tissues and their normal equivalents, as well as on the imbalance between expression of gelatinases and their native inhibitors, observed in cancer cells.

Present work deals with the comparison of proteolytic activity of gelatinases in eight cell lines, representing different types of cancer disease. Activities of cell sonicates have been determined by fluorometric assays, in the presence or absence of specific and non-specific inhibitors. The clue of the second part of experiment was to estimate changes of proteolytic potencial of cancer cells after treating with mild or strong detergent.

Significant differences between investigated cell lines were obtained, all in relation to overall proteolytic activity and to the susceptibility to inhibitors used. Consecutively, we observed diversified influence of detergent on studied cell lines. Particularly, by contrast to the others, application of mild detergent to black melanoma cell sample caused bigger increase of proteolytic activity than the disruption of cells with strong detergent.

P04-60
Colorectal cancer detection in rats using fluorescent fingerprint

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Among instrumental techniques, fluorescence spectroscopy is recognized as one of the most sensitive diagnostic tool with high efficiency compared to many routine medical diagnostic tools for many disorders diseases, especially for diagnosis of early cancer stage. Many metabolic compounds like porphyrins and NADH are produced in excess amounts in cancerous tissues due to rapid metabolism of cancerous tissue. These compounds are responsible for fluorescence spectra, what can be used for diagnosis of cancer along with other parameters. Fluorescence spectroscopy of biomolecules is considered a promising method to discriminate normal tissue from malignant tissue at various sites. However, only few studies have been reported on the feasibility of exploiting fluorescence spectroscopy of biological fluids to characterize pathological changes usable in diagnostic oncology. The fluorophore composition of biological fluids is related to different metabolic pathways. Urine contains a variety of organic and inorganic compounds including a number of natural fluorescent metabolites. The analysis of fluorescence from a sample of urine without any added reagents can provide useful information. Experimentally induced tumors in laboratory rats provide opportunity for studying certain aspects of tumors that cannot be effectively studied in humans. Significant information on human colorectal cancer etiology or factors influencing it has derived from studies using dimethyl hydrazine (DMH) model that is one of the experimental models appreciated for its morphological similarity to human. In our experiment we investigated urine samples from rats with early stages of DMH – induced colorectal cancer using fluorescent spectroscopy. As a model of chemically induced carcinogenesis we used 18 rats in which was induced carcinogenesis using DMH at a dose of 21 mg/kg subcutaneously, a total of five times at weekly intervals, in the 2nd, 3rd, 4th, 5th, and 6th week of experiment. The alterations of colonic tissue which are considered as precancerosis were also confirmed histologically. Eighteen rats served as healthy control group. Urine samples were obtained by bladder punction *post mortem*. After urine centrifugation (10 min, 5000 rpm), the supernatant was relocated into a new test tube and diluted (via geometric progression) with ultrapure water to get set of 12 dilutions. To create fluorescent fingerprint, synchronous fluorescence spectra (deltaλ30 nm) of every dilution were measured. By comparing healthy rat urine fluorescence with the fluorescence of urine obtained from rats with cancer, significant and specific differences were detected ($p = 0.003$). The analysis of autofluorescence of urine by fluorescent

fingerprint could help in the clinical diagnostics of cancer although further research is required. Supported by CEEMP-ITMS: 26220120067 (100%).

P04-61
Effects of radioiodine therapy on extracellular matrix degradation in papillary thyroid carcinomas with/without autoimmune thyroid diseases

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Background: The thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue, after thyrotropin (TSH) stimulation, is considered the ideal treatment for papillary thyroid carcinomas (PTC) and papillary thyroid carcinomas associated with autoimmune thyroid diseases (PTC + AITD). We aimed to evaluate the effects of therapeutic irradiation with I-131 on imbalance between expression of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in PTC with/without AITD patients.
Methods: We selected 45 patients with PTC (6M/39F, mean age 44.2 ± 19.1 years) and 38 with PTC + AITD (3M/35F, mean age 38.2 ± 19.6 years), who received the same dose of I-131 (3.7 GBq). All patients had elevated serum levels of TSH (> 30 mU/l). PTC + AITD patients had positive titers of anti-Tg autoantibodies (TgAb). Peripheric blood samples were collected before and at 96 hour after I-131 administration. The serum levels of TSH, TgAb, transforming growth factor-beta1 (TGF-β1), MMP-9 and TIMP-1 were measured by ELISA.
Results: Before I-131 therapy, the MMP-9 and TIMP-1 concentrations of PTC + AITD patients (635.12 ± 370.03 ng/ml, 140.27 ± 33.76 ng/ml) were higher than those of the PTC patients (484.98 ± 377.50 ng/ml, 128.6 ± 43.38 ng/ml). We found that I-131 therapy of PTC + AITD patients was associated with an increase in titers of TgAb (1.18-fold), TGF-β1 (1.27-fold) and a decrease in MMP-9 (1.27-fold) and MMP-9/TIMP-1 ratio (1.24-fold). In PTC patients, the serum levels of TGF-β1, MMP-9 and MMP-9/TIMP-1 ratio decreased 1.03-, 1.72- and 1.79-fold after irradiation.
Conclusions: In PTC patients the blockade of TGF-β1 signaling by I-131 therapy has almost halved the imbalance between MMP-9 and TIMP-1 and this decrease may reduce tumor cell viability and migratory potential. In PTC + AITD patients, increased TgAb titers partially block the beneficial effect of I-131. These titers are associated with increased TGF-β1 concentrations and with a lower decrease of MMP-9/TIMP-1 ratio, after I-131 administration, than in PTC patients.

P04-62
RNase A strongly inhibits metastasis development through the alteration of miRNA profiles in tumor tissue and blood serum

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Recent data on the involvement of miRNA in regulation of tumorigenesis showed a great prospect for these molecules as a novel class of therapeutic targets and gave a new start for the study of ribonucleases as potential antitumor and antimetastatic agents.

Previously we have shown that the administration of ultra-low doses of RNase A to mice bearing Lewis lung carcinoma (LLC) inhibits metastases development by 60–90% and causes the retardation of primary tumor growth by 30%. It was found that the observed antitumor and antimetastatic effects of RNase A are accompanied by reduction of pathologically elevated levels of extracellular RNA and increase in ribonuclease activity of blood plasma of tumor-bearing animals. Here in order to search for possible molecular targets of RNase A miRNA fractions were isolated from the serum and tumor of C57/Bl mice with LLC received treatment with RNase A or without treatment. Libraries of these small RNAs were prepared and analyzed using the SOLiD V3.5 sequencing system. Sequencing data revealed that treatment by RNase A resulted in decrease of the levels of serum miRNAs and increase of levels of timorous miRNAs whose direct and indirect targets are mRNAs encoding proteins of cell adhesion (ITGB1-3, ITGA5, ITGAV, LOX), proteolysis (TIMP3, ADAM17), angiogenesis (VEGF-A, HIF-1A) as well as cell proliferation (PTEN, PHB, RAS). Obtained data give the evidence that antitumor and antimetastatic effects of RNase A is associated with alteration of miRNA profiles in tumor tissue and blood serum which regulate the tissue architectural changes associated with malignancy.

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P04-63
Lipid lowering therapy decreases LDL-S-homocysteinilation levels in chronic kidney desease patients

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Chronic kidney disease (CKD) patients are characterized by diabetes, hypertension, obesity and dyslipidemia with a highly atherogenic profile: increased total and LDL cholesterol, triglyceride, and a decreased HDL-cholesterol. Moreover, elevated levels of LDL-homocysteinilation has been found in these patients and it is reported that this modification increase LDL-atherogenicity. The dyslipidemia control through lipid lowering therapy is one of the targets for the treatment of CKD. By this pilot study we aimed to evaluate the effect of hypolipidemic drugs on the levels of low molecular weight thiols bound to LDL in nephropatic patients. We enrolled thirty CKD randomized to receive three different hypolipidemic regimens: simvastatin alone (40 mg/day) or ezetimibe/simvastatin combined therapy (10/20 or 10/40 mg/day). Considering that proteins are thiolated in response to oxidative stress, evaluation of free malondialdehyde plasma levels, allantoin/uric acid ratio (All/UA) was also performed to monitor OS in patients during drug treatment. LDL thiolation decreased in all treated patients, but a greater efficacy was attained from a combined therapy with a higher simvastatin dose, by which a 31% decrease of all S-bound thiols was reached after one year of therapy. In particular, in this patients group the reduction of apoBHcy was > 40%. The concomitant decrease of the oxidative stress indices during the therapy brings to the hypothesis that decreased levels of protein bound thiols may be a consequence of oxidative stress improvement. Therefore, among the several beneficial effects described for lipid lowering drugs we also propose their ability to reduce the quantity of LDL linked homocysteine thus decreasing not only LDL levels but also LDL atherogenicity.

P04-64
Prognostic significance of ERCC1, RRM1 and BRCA1 in surgically treated non-small cell lung cancer patients

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Nowadays there is an interest in estimation of response rate to chemotherapy by predictive molecular markers. The aim of our study was to assess relation of mRNA levels of DNA repair genes ERCC1, RRM1 and BRCA1 in surgically removed tumor tissue to disease free interval (DFI) and overall survival (OS) in a group of 59 patients with non-small cell lung cancer NSCLC who had undergone curative lung resection and the adjuvant chemotherapy. Further, to investigate if potential residual tumor cells after resection reflect properties of a primary tumor and respond to chemotherapy according to level of predictive markers with the respect to current knowledge. Quantitative estimation of mRNA of selected genes in paired (tumor and control) lung tissue samples was performed by RT real-time PCR. We found lower mRNA expression of ERCC1 ($p < 0.001$) and RRM1 ($p = 0.023$) in NSCLC tumor tissue compared to normal lung tissue. Comparing expression levels in histological subtypes we recorded higher mRNA expression of ERCC1 ($p = 0.021$), RRM1 ($p = 0.011$) and BRCA1 ($p = 0.011$) in adenocarcinoma than in squamous cell carcinoma. We found longer OS in adenocarcinoma patients with higher expression of RRM1 mRNA ($p = 0.002$). In squamous cell carcinoma patients the longer OS was connected with higher expression of BRCA1 mRNA ($p = 0.041$). In NSCLC patients of stage 3 we found longer DFI in patients with higher expression of RRM1 ($p = 0.004$) and ERCC1 ($p = 0.038$). Patients who had been treated with adjuvant chemotherapy and had shown lower expression of repair genes had adverse prognosis. We observed that the assessment of DNA repair gene level in primary tumor treated by surgical resection had prognostic significance and did not predict response to adjuvant chemotherapy. Supported by the grant SVV-2012-264806.

P04-65
Study of the lipofecting activity of polyprenyltrimethylammonium iodides (PTAI): efficient transfection of various cell types with negatively charged serum compatible lipoplexes

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One of the major challenges facing the development of gene therapy is lack of efficient and safe gene vectors. In this study we tested a novel cationic polyprenyl derivatives as potential vectors for gene transfer.

Polyprenols were extracted from plant tissues and crude lipid extracts were subjected to the alkaline hydrolysis. Single prenologues were isolated chromatographically and PTAI were synthesized via phthalimide and amine. The size and zeta potential of PTAI-based lipoplexes were determinated. Cytotoxicity was evaluated by FDA and EtBr test and antimicrobial activity by colony reduction assay. *In vitro* transfection activities of lipoplexes were tested by GFP-expression assay using 11 normal and cancer cell lines and four primary human and mouse cell types both in the presence and absence of serum. Mechanism of lipoplex internalization and intracellular trafficking was examined using fluorescence microscopy and endocytosis inhibitors.

We have tested four derivatives: amino-Pren-7(AP-7), AP-8, AP-11, AP-15 in combination with DOPE, cholesterol, DC-cholesterol, DOPC, DSPE-PEG2000, PEG8000. The highest efficiency of transfection in the absence of serum (DU145 cells) was achieved for AP-11 + DOPE + pEGFP-C1 lipoplexes – 79%, without any toxic effect, while in the presence of serum multicomponent lipoplexes (PTAI + DOPE + DC-cholesterol + DOPC + pEGFP-C1) were most effective – 59%. The representative size of the most active AP-11 + DOPE + pEGFP-C1 lipoplexes was 96.71 ± 0.94 nm. Interestingly, they occurred to be negatively charged (−32.7 ± 1.65 mV). Moreover, some PTAI-based liposomes exhibited potent bactericidal activity against *S. aureus* and *E. coli*, while they showed no toxic effect on eukaryotic cells.

In conclusion, PTAI are effective lipofecting agents and PTAI-based multicomponent serum compatible lipoplexes may be promising candidates for *in vivo* gene delivery.

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P04-66
Folate-targeted submicrohydrogels as 5-Fluorouracil delivery system: *in vitro* and *in vivo* evaluation

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Folate has been extensively investigated for targeting various tumor cells overexpressing folate receptors. Folate-targeted drug delivery systems show a high affinity for the folate receptor, which enables transportation of the conjugate via receptor mediated endocytosis. 5-Fluorouracil (5-FU) is one of the chemotherapeutic compounds most used for the treatment of a great variety of tumors such as colorectal cancer, breast cancer, pancreatic cancer, or gastric cancer. Folate-targeted poly[(*p*-nitrophenyl acrylate)-*co*-(N-isopropylacrylamide)] nanohydrogel (F-SubMG) was loaded with 5-fluorouracil (5-FU) to obtain low (16.3 ± 1.9 μg 5-FU/mg F-SubMG) and high (46.8 ± 3.8 μg 5-FU/mg F-SubMG) load 5-FU-loaded F-SubMGs. The complete *in vitro* drug release took place in 8 hour with two stage drug release and different rates. Unloaded F-SubMGs are not toxic to MCF7 and HeLa cells *in vitro* up to high concentrations, and 5-FU-loaded F-SubMGs present effective elimination of carcinoma cells. The presence of folate in the submicrogels enhances their internalization in HeLa cells, which are receptor folate positive. In the case of MCF7 line, it is mainly produced by a nonspecific mechanism. Moreover, subcutaneous injection of F-SubMGs does not cause an acute inflammatory response or

rejection signs. Subcutaneous drug administration by 5-FU-loaded F-SubMG increases the mean residence time of drug. Therefore, the developed non-toxic folate-conjugate submicrogels have high potential to control the release of 5-FU, which may lead to a new option for treating several cancer malignances.

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P04-67
Activation of AP-1 protein and related increase of matrix metalloproteinase expression in hypercholesterolemia induced atherosclerosis

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Atherosclerosis and its complications are major causes of the death all over the world. One of the major risks for atherosclerosis is hypercholesterolemia. During atherosclerosis, lipoproteins such as LDL become trapped at the site of lesion where they converted to oxLDL.

OxLDL can stimulate inflammatory speciality of monocytes by increasing the MMP production. MMP's play an important role in atherosclerosis by degrading the extracellular matrix, which results in cardiovascular remodeling. Of them, MMP-1 degrades type I and III collagen MMP-9 degrades type IV collagen, the major matrix components in atherosclerotic plaques and released by macrophages, smooth muscle cells and endothelial cells. Studies show that, CD36-mediated activation of MAP kinase JNK1 is regulated by OxLDL. It is known that activation of transcription factor AP-1 plays a key role on MMP mRNA expression which may be generated by reactive oxygen species. Addition to this in atherosclerosis process, PPARγ has many antiinflamatory effects and also known to inhibit MMP expression with AP-1 pathway.

In the present study we elucidated *in vivo* molecular mechanisms of atherosclerosis induced by hypercholesterolemia and the role of Vitamin E. Therefore we investigate c-jun, JNK1, MMP-9, MMP-1 and PPARγ mRNA expressions and phospho c-jun, c-jun, JNK1, PKC, MMP-9, MMP-1 protein expressions in rabbit aorta. By this way our aim was to identify the signaling molecules/transcription factors are involved during the progression of atherosclerosis after CD36 activation.

Our results suggest that hypercholesterolemia effects c-jun expression at the transcriptional and/or post-transcriptional level. With this activation, the MMP expression increases and this results in an improvement of atherosclerosis. At this step we observed that vitamin E has an inhibitory effect.

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P04-68
Abnormal glycogen is harmful to cells

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Glycogen stores make a critical contribution to the normal functioning of cells and the organism as a whole. An over-accumulation of glycogen, often in the form of poorly branched glycogen that is resistant to degradation, referred to as polyglucosan bodies, leads to dysfunction in many tissues. It is relevant to note that different glycogenoses like Lafora Disease and Andersen Disease, both lead to the formation of 'abnormal' glycogen accumulation, although they are caused by mutations in different genes involved in the glycogen-metabolism pathway. Moreover, the clinical manifestations vary between the two disorders. Our results highlight the requirement of normally branched glycogen for proper development, since the absence of the GBE-1 protein in mice was lethal at embryonic stages as early as day E10.5 and adult GBE-1 heterozygous mice showed accumulation of polyglucosan bodies in the cerebellum, cortex and hippocampus. We propose that Glycogen Branching Enzyme 1 (GBE-1) is a crucial protein in the process of regular glycogen storage and its demise impairs cell viability and function from embryonic stages onwards.

P04-69
Immunohistochemical analysis of caveolin-1 in follicular cell derived thyroid gland tumours

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Thyroid gland tumours represent one of the most common endocrine neoplasms; its hystotipes are difficult to differentiate and treat accordingly, therefore accurate diagnosis is crucial. Recent data suggest that the expression of caveolin-1, a major constituent of caveolae, may vary with the type of the thyroid tumour, giving it a potential to differentiate certain thyroid tumour counterparts. We aimed to evaluate caveolin-1 expression by means of immunohistochemistry on 116 cases of thyroid tumours, including papillary and follicular carcinomas and follicular adenomas and used Western immunobloting to reveal the expression patterns on 4 tissue homogenates of papillary carcinoma. Statistical analysis included Mann Whitney U test for comparison between groups. Classical papillary carcinoma was positive in 93.9% of cases, whereas follicular variant of papillary carcinoma was positive in significantly less number of cases i.e. 85.3% ($p < 0.005$). Follicular carcinomas were positive in 52.6% of cases and follicular adenomas exhibited immunostaining in only 16.7% of cases. Adjacent peritumoral tissue was mostly negative giving 17.8% of positive cases. Significant differences were observed between the three hystotypes whose differential diagnostics is difficult, i.e. follicular carcinomas and adenomas ($p = 0.009$), follicular variant of papillary carcinoma and follicular carcinoma ($p = 0.000$) or adenoma ($p = 0.011$). Western immunoblotting using four cases of papillary carcinoma and adjacent peritumoral tissue homogenates confirmed expression patterns demonstrated by immunohistochemistry. Our results demonstrated that analysis of caveolin-1 expression in thyroid tumours might be helpful in distinguishing between thyroid histological counterparts.

P04-70
Novel optical properties of lipid-coated nanoparticles for molecular imaging

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The application of optical nanoparticles in biomedical research is increasing because of their high spatiotemporal resolution and high sensitivity in the imaging platforms. However, until now, the biocompatibility issues of these optical nanoparticles should be resolved before future clinical use. Lipids are good natural insulators for biological compartmentation. It also preserved the inert properties to reduce unwanted redox reactions in cells. Assembly of synthetic lipids on the surfaces of optical nanoparticles has been successfully achieved in our laboratory. These lipid-coated nanoparticles have been used in tracking nanoparticle distribution in an animal model. Metal nanoparticles have been approved to enhance contrast in magnetic resonance imaging (MRI), but not to be efficient in optical systems. We report a series of lipid-coated metal nanoparticles that was designed to have strong third harmonic generation (THG) signals. Using cationic lipids as part of the shell, these lipid-coated nanoparticles entered into cells efficiently without observed cytotoxicity. The labeled tumors in mice could be detectable by two noninvasive imaging systems, MRI and THG, simultaneously. This is the first time to use such nanoparticles as THG contrast agents *in vivo*, by which the high resolution images of tumors and its environments are directly obtained. Our study demonstrated a possibility of using multifunctional lipid-coated nanoparticles for molecular imaging and cell tracking.

P04-71
Isolation of canine C-reactive protein (CRP) and development of polyclonal antisera for CRP analysis in sera

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Acute phase proteins (APPs) have been used as biomarkers of inflammation, infection and trauma in human medicine. Similarly, the possible use of the acute phase proteins in veterinary medicine, requires the availability of automatable immunochemical methods of measurement. The aim of this study has been focused in CRP being this protein the major APP in dogs. CRP has been isolated from dog acute phase sera, using an Immobilized *p*-Aminophenil Phosphoryl Choline Gel. This isolation procedure is based on the Ca^{2+} dependent affinity of CRP for the phosphorylcholine. Canine CRP is composed of five subunits organized in a cyclic pentameric disc structure as described for proteins of the pentraxin family. In SDS-PAGE, isolated CRP shows two bands of around 24.5 and 22.2 kDa. The nature of these two bands could be related to glycosylation in two of the five subunits in each CRP molecule. Antisera to dog CRP were raised in rabbits by subcutaneous injections of purified protein, and adsorbed with insolubilized normal dog serum. The antisera obtained were specific to the CRP and showed a high antibody titers (3.19 ± 0.05 mg/ml). The CRP concentration in canine serum was determined by single radial immunodiffusion (SRID). In healthy dogs sera, CRP levels were lower than 10 μg/ml. However, in dogs with different pathological processes CRP concentrations increased to values ranging from 95 to 450 μg/ml. As summary, the antisera generated in this work are useful for future immunochemical methods for canine CRP determination.

P04-72
Bioenergetic changes in mitofusin deficient cells. Possible implication in CMT2A pathology

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Mitofusin 2 (Mfn2), mitochondrial outer membrane protein which is involved in its rearrangement, was first described in pathology of hypertension and diabetes, but nowadays much attention is paid to its functions in Charcot-Marie-Tooth type 2A neuromiopathy (CMT2A). However, little is known about the mechanism of axonal damage due to Mfn2 mutations and the role of Mfn1 in this process. In our studies we investigated the effects of Mfn2 deficiency on cell metabolism in the presence or absence of Mfn1. Experiments were performed on three lines of Mouse Embryonic Fibroblast (wtMEF, $MEF^{Mfn2-/-}$ and $MEF^{Mfn1-/-Mfn2-/-}$). We observed the reduction in maximal cell respiration in KO-cells, which was accompanied by significant decrease in complex I and IV subunits content in double knockouts, and same trend in $MEF^{Mfn2-/-}$. Despite that, total ATP content was the same in all cell lines tested. Interestingly, an inhibition of mitochondrial ATP-ase with oligomycin in the absence of glucose in the medium did not significantly reduced ATP content in $MEF^{Mfn1-/-Mfn2-/-}$. It suggests more pronounced participation of glycolysis in ATP synthesis in these cells that found in the wild type ones. It correlates with substantially higher amount of glycogen stored in KO cells in comparison to wt MEF. Therefore, it seems that mitofusins deficiency causes a shift in metabolism from oxidative to anaerobic. It may be an important feature of CMT2A pathology and the contribution of Mfn1 shouldn't be omitted. The project is supported by NSC grant NN402474640.

P04-73
Seven-membered cycloplatinated complexes as a new family of anticancer agents

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A series of seven-membered cyclometallated Pt(II) complexes containing a terdentate [C,N,N'] ligand (1a-1c and 2a-2c) have

been developed as potential monofunctional DNA binding agents. By reactions of cis-[Pt(4- -SEt2)]2 or cis-[Pt(C6H5) 2(SMe2)2] with imines 2-ClC6H4CH = NCH2CH2NMe2 (b) or 2-F,6-ClC6H3CH = NCH2CH2NMe2 (c) the new compounds 1b, 1c and 2c were synthesized and characterized. Complex 1b and 1c were further characterized by X-ray crystallography. The cytotoxicity assessment of the seven-membered platinacycles 1 (1a-1c) and 2 (2a-2c) against a panel of human cancer cell lines (A549 lung, HCT116 colon, and MDA MB231 breast adenocarcinomas) revealed that the six cycloplatinated complexes exhibit a remarkable antiproliferative activity, even greater than cisplatin in the three human cancer cell lines. From a pharmacological point of view, platinacycles 1 (1a-1c) and 2 (2a-2c) may represent compounds for a new class of antitumor drugs. Electrophoretic DNA migration studies showed that all of them modify the DNA tertiary structure. Induction of S-G2/M arrest and apoptosis were also observed for one of the representative compounds (1c) of the series.

P04-74
CCR5 and CXCR4 coreceptors may not be responsible for conferment of resistance to HIV exposed but seronegative individuals of Nigerian origin

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Introduction: A mutant allele of CCR5 with 32-base-pair deletion has been found in the highly-exposed group of seronegative individuals who are naturally resistant to HIV infection in European populations. This mutation presumably does not occur in African populations hence the high prevalence of HIV in the region. This study therefore, seeks to ascertain the implication of HIV-coreceptor expressions in HIV/AIDS resistance using serodiscordant-heterosexual partners of Nigeria origin.
Methods: Thirty-four partners (serodiscordant-seronegative{SSN}and serodiscordant-seropositive {SSP}) and 15 seronegative-healthy individuals(SNH) were recruited for the study. HIV was confirmed using immunecomb-II. FACScan flow cytometer was used to measure CD4,CD3,CD8,CCR5 and CXCR4 cell expressions. NucliSens magnetic extraction method based on Boom chemistry was used for HIV-mRNA extraction while real-time quantification was done by Nucleic Acid based amplification and detection assay(NASBA).
Results: We noted a significantly increased T-cell ratio in SSN group by 40% on comparism with SSP. HIV-mRNA was not detected in SSN and SNH but was highly expressed in the SSP group(9400 ± 700). Expression profile of the co-receptors showed that SSN's CCR5(800 ± 45) and CXCR4(756 ± 80) decreased non-significantly($p < 0.05$) by 7.5% and 9% respectively when compared with SSP. Similarly, expression of CXCR4(876 ± 65) and CCR5(900 ± 152) in SSP increased slightly over SSH. SSP, SSH and SSN groups did not show any significant difference in their chemokine expression patterns.
Conclusions: Since cytokine-mediated increase in binding of HIV to cells is related to increased expression of CCR5 and CXCR4 our results therefore, preliminarily indicates that the HIV-coreceptor mutation may not be a factor in conferment of resistance to the SSN group.

P04-75
Discovery of new hits for TRPV1 blockade by high throughput assays

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Although pain is a normal and necessary alarm/defense mechanism for life, chronic pain, either inflammatory or neuropathic, is a pathologic process for which there is not still an adequate treatment to avoid it. In the recent years an ion channel called TRPV1 belonging to the family of the Transient Receptor Potential (TRP) channel has been related with this chronic pain. Pharmacological blockade and genetic deletion on TRPV1 experiments, has validated it as a therapeutic target, generating intensive drug discovery programs aimed at developing orally active antagonists. Consequentially, numerous TRPV1 antagonists have been identified that block the receptor with high efficacy and potency. However, and rather disappointingly, despite the claimed therapeutic potential of these TRPV1 antagonists, very few candidates have progressed into clinical trials because of unpredicted side effects such as hyperthermia. In this study were evaluated the biological activity of new chemical libraries, through high throughput screening. We report here the identification of compounds that presented a high blockade activity on TRPV1. This new pharmacophoric scaffold can be used as a hit for analgesic drug development targeting TRPV1.

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P04-76
The analysis of putative antiallergic potential of three lactobacillus strains in the global phenotypic approach

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The increase in incidence of allergy in the last few decades has been linked to the high hygienic standards connected to a reduced microbial stimulation of the mucosal immune system. There is much evidence concerning the role of probiotics in the prevention and therapy of allergic diseases. Various strains of lactic acid bacteria (LAB), especially lactobacilli and bifidobacteria, are considered as probiotics and have been reported to suppress the allergic reactions. Recently, two strains of *Lactobacillus casei* LOCK 0900 and LOCK 0908 and one strain of *Lactobacillus paracasei* LOCK 0919 were selected according to their antagonistic activity against pathogenic bacteria, tolerance to low pH and bile acids. Preliminary studies have shown that ingestion of that the mixture of *L. casei* LOCK 0900, *L. casei* LOCK 0908 and *L. paracasei* LOCK 0919 strains affects the immune system by inducing TH1 and regulatory cytokine production and by suppressing pro-allergic response.

In this work, we present an additional phenotypic characterization, as it is considered to be essential in better defining the role and use of specific probiotics Lactobacillus strains. Using Biolog Phenotype Microarrays we investigated, which of metabolic substrates (various carbon and nitrogen sources) could or could not be used by these Lactobacillus strains. The same technique was

used to examine the response of tested strains to various osmolytes and pH.

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P04-77
Molecular imaging of experimental thyroid proliferative disorders

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Introduction: Positron emission tomography (PET) produces functional images for studying tumor behavior and for the follow-up of antitumoral therapies. TFB (^{18}F-Tetrafluoroborate) is a new PET tracer binding to sodium-iodine symporter (NIS) which mimics iodine behavior and may be used for healthy thyroid and tumor imaging. FDG (^{18}F-fluorodeoxyglucose) shows increased glycolytic activity observed in neoplastic cells. We analyzed the potential of the tracers TFB and FDG for the combined study of the progression of thyroid proliferative disorders. We have evaluated NIS gene expression levels in order to compare them to the results of TFB-PET.

Material and Methods: Wistar rats received a Thyroid-stimulating hormone stimulus treatment by 0.1 M potassium perchlorate solution *ad libitum* as drinking water. After 12 months, thyroid proliferation lesions started to appeared. MicroPET scans in Philips Mosaic, Bioscan nanoCT and PET Siemens Biograph mCT systems were carried out with TFB and FDG, and fused with CT. NIS mRNA expression from thyroid tissues was studied by SYBR Green quantitative RT-PCR (qRT-PCR). Histological analysis was performed.

Results: TFB-PET revealed highly specific capture in thyroids and lesions, although did not correlate exactly with the gland extent. For FDG-PET healthy thyroid and hyperplasias captured less than papillary thyroid cancer. NIS mRNA qRT-PCR revealed that NIS expression levels were higher in proliferative lesions than in normal thyroid, although they did not depend on thyroid gland size; hence it agreed with the results of the PET/CT studies.

Conclusions: When TFB and FDG are used, NIS expression can be merged with glucose consumption, making possible the discrimination between thyroid cancer and hyperplasia, which do not exhibit high glucose consumption.

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P04-78
Antitumoral effect promoted by amitriptyline in combination with camptothecin in a breast cancer cell line

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Introduction: Oxidative therapy is a promising anticancer strategy based on the production of high levels of ROS and/or the depletion of antioxidants of tumor cells. In this study we have focused on the potential antitumor activity of amitriptyline (Amit), a tricyclic antidepressant, in combination with the chemotherapeutic drug camptothecin (CPT) in a human breast cancer cell line.

Materials and Method: MCF-7 cells were treated 24 hour with Amit (20 and 50 μM), 10 μM CPT and a combination of both. Apoptosis was analyzed by observing nuclei fragmentation with Hoechst staining. Generation of ROS was studied using dihydrorhodamine-123 and flow cytometry. We made western blotting of complex III. Activity of mitochondrial complexes was determined in cell extracts using spectrophotometric methods.

Results: Cell death was higher in the presence of Amit in comparison with CPT, especially significant at 50 μM, but less than with the combination of the drugs. The level of ROS in breast tumor cells treated with Amit was significantly increased respect to untreated cells in a dose dependent manner. The activity of mitochondrial complexes with the combination treatment was lower than the observed with Amit alone. Furthermore, complex III proteins decreased in both Amit and CPT treatments, but the decrease was more accused when tumor cells were treated with the combination of the drugs. This effect was also observed in ATP production.

Conclusions: Our results in breast tumor cells suggest that Amit induces alterations in mitochondrial activity and high level of ROS, inducing apoptosis. These effects were more pronounced in cells treated simultaneously with Amit and CPT. Amit is proposed to be tested together with different chemotherapeutic drugs in antitumor therapies.

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P04-79
Microsatellite instability analysis in bladder cancer in Egypt

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Background and aim: Bladder cancer is a common malignancy in Egypt. It is associated with history of Schistosomiasis infection. In the present study, microsatellite instability (MSI) has been evaluated as an early noninvasive marker for diagnosis bladder cancer in Egyptian patients.

Patients and Methods: The study included a total of 34 bladder cancer patients (admitted to NCI and TBRI hospitals) pathologically diagnosed, staged and graded. Exfoliated urinary cells taken from these patients and from 10 normal controls. DNA was extracted and polymerase chain reaction was done product was separated by 12% PAGE. Panels of five quasi-monomorphic mononucleotides (BAT-25, BAT-26, NR-21, NR-22 and NR-24) were evaluated.

Results: MSI in at least one marker was observed in 71.87% of bladder cancer cases .Mutations in BAT 25 (8.8%), BAT-26 (11.7%), NR-21 (52.9%), NR-22 (32.3%) and NR-24 (0%) were observed. Good association of MSI was seen with tumor stage and grade. All normal cases showed normal pattern with no observed mutations

Conclusions: MSI markers may be used as independent early diagnostic and also prognostic markers in cases of bladder cancer

P04-80
Sodium tungstate modulates inflammation of diabetic nephropathy

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Diabetes mellitus induces significant complications in human patients, among these diabetic nephropathy (DN). Sodium tungstate (Na2WO4) has been described as a potent normoglicemiante by exerting an insulin mimetic action. In the present study we investigated the renal effect of Na2WO4 and their anti-inflammatory mechanisms in a diabetic rat model. The effect Na2WO4 on DN was evaluated in streptozotocin-induced diabetic rats. After four months of diabetes, kidney damage was characterized by biochemical and anatomic pathology. Renal fibrosis in diabetic rats as well as treated diabetic rats were analyzed by staining of collagen type IV by immunohistochemistry and Masson trichrome. The ultrastructural alterations were evaluated by electron microscopy. Additionally, inflammatory damage was assessed by IHC, qRT PCR and Western blot of osteopontin. The pathologic analysis results showed a marked glomerular renal damage mainly to four months of diabetes. In animals treated with Na2WO4 we observed decrease fibrosis and inhibition of the inflammatory kidney damage detected by large decrease of the accumulation of collagen and decreased expression of osteopontin, respectively. In conclusion Na2WO4 is able to inhibits the expression of profibrotic and pro-inflammatory proteins characteristic of this disease suggesting that this drug could be effective treatment to prevent renal fibrosis in advanced stages of diabetic nephropathy. (Fondecyt 1090694).

P04-81
Telomere shortening and cell senescence induced by perylene derivatives in A549 lung cancer cells

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Cancer cells evade replicative senescence by re-expressing telomerase, which maintains telomere length and hence chromosomal integrity. Telomerase inhibition would render cancer cells to senesce and therefore prevents cancer cells to grow indefinitely. A G-quadruplex ligand can attenuate telomerase activity by inducing G-quadruplex formation at the 3'-overhang of telomere and at the human telomerase reverse transcriptase (hTERT) promoter; the former prevents telomerase to access to the telomere and the latter acts as a transcriptional silencer. Here we show that our perylene derivatives: PM2 and PIPER, induced G-quadruplex formation from both telomeric DNA and the hTERT promoter region *in vitro*. The TRAP assay showed that these compounds could inhibit telomerase in a dose-dependent manner. When A549 human lung cancer cells were treated with these compounds, hTERT expression were down-regulated. Moreover, the crude protein extract from these treated cells had less telomerase activity. We further demonstrated that treating A549 cells in a sub-cytotoxic dose for a long period of time resulted in a telomere shortening and cell senescence. This study might provide the foundation for the development of perylene derivatives as effective agents for cancer therapy.

P04-82
Treatment of BT-20 cells with anticancer agents revealed alterations in the expression pattern of the apoptosis-related genes *BAX, BCL2* and *BCL2*: new promising indicators for predicting chemotherapy response in breast cancer

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BCL-2 family members have been studied in breast cancer as prognostic biomarkers and as new potential targets for molecular therapy. In the present study, the purpose was to discover the utility of *BAX, BCL2* and *BCL2L12* genes as putative indicators of breast cancer treatment. Changes in the mRNA expression of the pre-mentioned genes were investigated after treatment of BT-20 cells with epirubicin, docetaxel and methotrexate. MTT and trypan blue assays were used to assess the cytotoxic effect of each drug. The relative quantification of *BAX, BCL2* and *BCL2L12* mRNA levels was performed by using the SYBR Green® I chemistry. *HPRT1* served as an endogenous control gene. Additionally, we developed an immunocytochemical methodology, so as to affirm that the cells died through the apoptotic pathway under the action of the different drugs. Following the direct development of treated and untreated cells onto slides, the expression of various apoptotic proteins was studied. Distinct modulations in the expression of the above genes are induced as a response of the cells to the administration of each drug. The most significant alterations were a 4-fold and 3-fold decrease of *BCL2* in epirubicin and docetaxel treated-cells, respectively, compared to the untreated ones. In the case of *BAX*, its levels increased by 2-fold in methotrexate treated-cells. As regards the mRNA expression of *BCL2L12*, a 2.5-fold and 2-fold increase were noticed in epirubicin and methotrexate treated-cells, respectively, whereas these levels decreased by 2-fold in docetaxel treated-cells. Our results indicate the differential expression of *BAX, BCL2* and *BCL2L12* after exposure of breast cancer cells to anticancer drugs and may provide evidence for their utility as promising predictive biomarkers of breast cancer cells' response to treatment.

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P04-83
Can CD36 expression in peripheral blood mononuclear cells be use for evaluation of atherosclerosis in hypercholesterolemic rabbits?

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Atherosclerosis, characterized by the accumulation of plasma lipoproteins that carry cholesterol and triglycerides in the arteries, is one of the major causes of morbidity and mortality worldwide. In the atherosclerotic process, following the penetration of phagocytic monocytes into the subendothelial space, they are differentiated into macrophage foam cells with the rapid transformation. Meanwhile, atherogenic lipoproteins which are modified low density lipoprotein (LDL) are up taken by receptor-mediated endocytosis mechanism. Cluster of differentiation 36 (CD36) is one of important scavenger receptor playing role in atherosclerotic process. CD36 mediates intake of ox-LDL by macrophages in the arteria walls and long chain fatty acids into the cells.

In the present study, we investigated CD36 mRNA expressions in both aortic tissues and peripheral blood mononuclear cells compared to each other and the effects of vitamin E on these changes in the atherosclerotic rabbit model induced by 2% cholesterol containing diet. In the cholesterol group, significant increase in the CD36 mRNA expressions of rabbit aorta and peripheral blood mononuclear cells was observed compared to control group and in the vitamin E treated group a significant decrease was observed. Our results demonstrated that the CD36 mRNA levels in peripheral blood mononuclear cells reflect the levels in aorta. Our results show that CD36 expression in peripheral blood mononuclear cells might be used as a marker for detection of atherosclerosis.

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P04-84
ROS and antioxidant levels of normal and neoplasic thyroid cells are affected differently by amitryptiline in oxidative therapy

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Introduction: Oxidative therapy is an anticancer strategy based on the generation of high levels of oxidative stress and/or by depleting the protective antioxidant machinery of tumor cells. We have already described that amitriptyline (Amit), a tricyclic antidepressant, increases ROS levels and decreases the level of some antioxidants in a lung cancer cell line. We focused this work on the potential capacity of Amit to stimulate ROS production and downregulate antioxidant status in human thyroid cell lines, comparing the effect of Amit in normal and neoplastic cells.
Material and methods: The following human follicular-cell thyroid cell lines were used: Nthy-ori3-1 (normal thyroid), FTC-133 (follicular thyroid cancer), 8505C (anaplastic thyroid cancer). Cell cultures were treated with Amit (0, 10, 50 μM) for 24 hour. Antioxidants (catalase, MnSOD, glutathione reductase) mRNA expression was studied by SYBR Green quantitative RT-PCR (qRT-PCR). Generation of ROS was analyzed using dihydrorhodamine-123 and flow cytometry.
Results: The level of ROS in thyroid tumor cells treated with Amit was significantly increased respect to untreated cells in a dose dependent manner, and it was higher in tumor cells than in normal thyroid cells. 50 μM Amit induced a decrease in the antioxidants expression levels of all tumor cells. However, when tumor cells were treated with 10 μM doses, cells responded rising antioxidant levels as a defense against increased ROS. In normal thyroid cells, low doses of Amit induced higher increase of antioxidant than tumor cells, and high doses of the drug limited the cells to respond against higher ROS.
Conclusions: Although caution must be taken when using 'normal' cells from permanent cell lines, our results suggest that non-tumoral thyroid cells have less oxidative stress levels and a higher capacity of resistance to oxidative insults than tumoral thyroid cells. So, Amit should be evaluated as a new anticancer drug for thyroid cancer marked by oxidative therapy strategy.
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P04r-85
Liposome nanoparticles for targeted drug delivery, gene delivery and magnetic imaging

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Liposomes are spherical vesicles with an aqueous inner cavity surrounded by lipid bilayer membrane. Liposomes of around 100 nm mean diameter can be classified as nanoparticles (liposome nanoparticles, LNP). LNP exhibit several properties such as ultra small size, large surface area to mass ratio, and high reactivity which may be useful in various applications. The diameter of the LNP is an important attribute enabling them to pass various *in vivo* barriers for systemic delivery. Investigations aimed at developing multifunctional LNP to deliver drug and gene with peptide driven targetibility and MRI visualization using the inner ear as a model target organ for developing therapies of inner ear disorders.

As a 1st aim, the main effort has been given to prepare LNP with a diameter less than 100 nm using a novel procedure, adaptive focused ultrasound (AFU). AFU has several advantages compared to other techniques as it is non-invasive, isothermal and the energy involved is much more precisely controlled due to focusing of the acoustic energy.

Second aim of this project was to analyze the efficacy of the penetration of LNP through the round window membrane when injected into the middle ear cavity in mice and to determine whether LNP accumulating in the inner ear tissues was sufficient to elicit a therapeutic response. Our results demonstrate that LNP are capable of carrying a drug (Disulfiram) into the inner ear that elicits a biological effect, with consequences measurable by a functional readout.

Under 3rd aim the study investigated for selective targetability of inner ear neuronal cell survival receptor TrkB for targeted gene delivery. We demonstrate the feasibility of targeting of LNP to TrkB expressing cells by designed peptides, promoting cellular uptake via receptor-mediated pathways.

Our 4th aim was to track the dynamics and distribution of LNP *in vivo* by preparing MRI traceable LNP. Effective MRI traceable LNP were developed by encapsulating Gadolinium which were visualized *in vivo* in the rat inner ear using a 4.7 T MR machine and the dynamics correlates to the status of the perilymph circulation.

P04-86
Estrogen improves vascular function in preeclampsia via ROS reduction

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Preeclampsia is a multisystemic disorder of pregnancy and characterized by hypertension and proteinuria in the second half of gestation. Preeclamptic women have extensive systemic vascular infiltration of neutrophils, which release reactive oxygen species (ROS) that might enhance vascular reactivity to vasoconstrictor and decreased bioavailability of NO. Estrogens as hydrogen donors from their phenol-hydroxyl ring have antioxidant effects. In the present study, we evaluated changes of ROS and reactive nitrogen species concentrations in plasma and value of blood pressure during short-term estradiol therapy in preeclampsia and healthy pregnancy. Intramuscular injections of 10 mg 17b-estradiol were administrated to 20 healthy and 20 preeclamptic pregnant women during three days. Analyses of mean arterial pressure (MAP) values, estradiol concentrations, superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and peroxynitrite ($ONOO^-$) were performed before and during the therapy. The results of our research indicated higher plasma concentrations of O_2^-, H_2O_2 and NO_2^- in preeclampsia compared to healthy pregnancy. There were no differences in plasma concentrations of $ONOO^-$ and estradiol serum. During estradiol therapy, were found the increased concentration of estradiol and decreased concentrations of O_2^- and $ONOO^-$ in healthy pregnant women. Estradiol therapy decreased MAP, O_2^-, H_2O_2, NO_2^- and $ONOO^-$ in preeclampsia. Concentration of estradiol was not significantly different before and during estradiol therapy in preeclamptic women. We conclude that reduction of MAP in preeclampsia during estradiol therapy is attributed to reduction of reactive oxygen species by estradiol, which leads to decrease of vascular reactivity and increase of NO bioavailability.

P04-87
Modification of biosensors surfaces with enzymes and antibodies for biochemistry and medicine – immobilization and analysis methods

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Preparation of well defined biosensing surfaces for screening and diagnostic purposes is a great challenge in the advanced biosensors techniques (ISFET, microcantilevers, electrochemical impedance, atomic force microscopy). Monitoring of an immobilization of molecular receptors (small molecules, proteins) on the sensor surface is crucial for the activity of the final device. Here we present several protocols for biomolecules immobilization on the Au and Si/SiO_2 surfaces as well as the analytical techniques for the evaluation of the process. Moreover methods for surface deactivation are also developed.We tested silanization of Si/SiO_2 surface using alkoxysilane derivatives with amine or epoxy functional groups. Au surface modification based on thiol chemistry (cysteamine, 11-mercaptoundecanoic acid) was also investigated.

Some methods of functional groups activation for protein binding were evaluated including glutaraldehyde and *N*-hydroxysuccinimide (NHS) chemistry. Additionally we compared the covalent immobilization of proteins on the Si/SiO_2 and Au surface with the unspecific, non-covalent interaction of macromolecules with the surface.Surface deactivation methods for prevention of unspecific protein binding to the Au or Si/SiO_2 surfaces were also analyzed. β-mercaptoethanol or poly(ethylene glycol) methyl ether thiol were used for Au surface and ethanolamine or methyl-terminated poly(ethylene glycol) compound activated as NHS ester were employed for Si/SiO_2 surface. These methods were compared with surface deactivation with non-covalent adsorption of neutral proteins i.e. BSA. Immunoenzymatic tests, X-ray photoelectron spectroscopy and atomic force microscopy were used for evaluation of immobilization methods used.

P04-88
Membrane the thermosensor which interacts with stress proteins

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Important diseases are known to be associated with abnormal stress (or heat shock) protein (HSP) levels and characteristic membrane defects. The present study aims to establish a mechanism for the possible interconnection between specific changes of lipid composition, fluidity- and microdomain organization of plasma membrane and the simultaneously altered (dysregulated) expression of HSPs. Exposure of cells to non-proteotoxic membrane fluidizers or non-proteotoxic drug candidates which interact specifically with certain membrane domains can strongly modulate the expression of HSPs. Monitoring the surface membrane microdomains by confocal- and ultrasensitive single molecular microscopy we established a relationship between specific distribution of lipid nanostructure ('rafts') and the concomitant changes in the level, profile and cellular distribution of HSPs. A comparative lipidomics study explored key lipid molecular species with the potential to activate of HSP signaling pathways. Drug candidates, capable to refine HSP profile by targeting specific membrane microdomains, – with considerable therapeutic benefit -, will also be discussed. A subpopulation of HSPs is membrane associated: via their specific lipid interactions these HSPs can control major attributes of the membranes like fluidity or curvature. The membrane microdomain associated HSPs can also participate in the orchestration of distinct raft-associated signaling platforms: thus, membrane association of HSPs can refine *hsp* gene expression.

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P04-89
Screening of pharmacological treatments for MELAS syndrome using cellular models of the disease

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MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) is a mitochondrial disease most usually caused by point mutations in tRNA genes encoded by mitochondrial DNA (mtDNA). Approximately 80% of cases of MELAS syndrome are associated with a m.3243A > G mutation in MT-TL1 gene, which encodes the mitochondrial tRNA-Leu(UUR). Currently, no efficient treatments are available for this chronic progressive disorder. Treatment strategies in MELAS and other mitochondrial diseases consist of several drugs that diminish the deleterious effects of the abnormal respiratory chain function, reduce the presence of toxic agents or correct deficiencies in essential cofactors. In this study we evaluated the effectiveness of some common pharmacological agents which have been utilized in the treatment of MELAS, in yeast, fibroblast and cybrid models of the disease. The yeast model harbouring the A14G mutation, in the mitochondrial ARNtLeu (UUR) gene, which is equivalent to the A3243G mutation in humans, was used in initial screening. Next, the most efficient drugs that were able to rescue the respiratory deficiency in MELAS yeast mutants were tested in fibroblasts and cybrid models of MELAS disease. According to our results, supplementation with riboflavin or coenzyme Q10 effectively reversed the respiratory defect in MELAS yeast and improved the pathologic alterations in MELAS fibroblast and cybrid cell models. Our results indicate that cell models have great potential as a screening and validation assays of novel drug candidates for MELAS treatment and presumably also for other diseases with mitochondrial impairment.

P04-90
Correlation between liver enzymes and free fatty acid levels in patients with type 2 diabetes

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It has long been proposed that elevation of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) and increase in free fatty acids (FFAs) levels may be associated with insulin resistance (IR) and Type 2 *diabetes mellitus* (T2DM). Limited number of studies have examined the association of liver enzymes and FFAs with T2DM control. Up to now, synchronized data related to the relevance of continuous monitoring of these markers in progression of T2DM are lacking. In this study we examined association of liver enzymes activities and FFA (saturated, SFA and unsaturated, UFA) levels in T2DM patients. We analyzed the activities of ALT, AST (aspartate amino transferase), GGT and FFA (palmitic, C16:0; stearic, C18:0 and linoleic acid, C18:2) levels, fasting plasma glucose (FPG) in 40 T2DM patients, and 40 age-matched healthy controls. Blood samples were collected from all participants in regular 3-months intervals up to 6 months period. All subjects included in this study were free of evidence of hepatitis, viral infection, or active liver and kidney damage. Standard IFCC enzyme protocols were used to determine enzyme activities on the Alcyon analyzer, while concentrations of palmitic, stearic and linoleic acid were determined by gas chromatography. As espected, the results showed a significant correlation was observed between liver enzyme activity and palmitic, stearic and linoleic acid levels in Type 2 diabetic patients ($p < 0.05$). In all three time intervals of measurements a highly positive correlation between AST, ALT and GGT and palmitic acid, ALT and GGT with stearic and linoleic acids while, a negative correlation was observed between AST and stearic and linoleic acids. Interestingly, significant correlation was observed between stearic acid and GGT activity in diabetic patients ($p < 0.05$). Our data suggest relevance of monitoring of sinchronous monitoring of liver enzymes and FFA levels, particularly GGT and stearic acid, in even adequately controlled diabetes.

P04-91
A new gas chromatography – mass spectrometry (GC-MS) methodology to evaluate the 5α-reductase activity

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The incidence of prostate cancer has significantly increased in the recent years. Although androgens are required for the function and integrity of prostate tissue, they can be implicated in prostate cancer and benign prostatic hyperplasia (BPH). Therefore, the reduction of androgen levels can be a valuable strategy for the treatment of prostate cancer by targeting 5α-reductase, the enzyme responsible for the irreversible conversion of testosterone (T) into its more active metabolite dihydrotestosterone (DHT), with 5α-reductase inhibitors (RIs) like finasteride. This work refers to the development and validation of a new method based on a dispersive liquid-liquid microextraction (DLLME) procedure followed by gas chromatography – mass spectrometry (GC-MS), to evaluate the 5α-reductase activity, by measuring the conversion percentage of T into DHT. Enzymatic assays were carried out in human prostate microsomes, using T as substrate and NADPH as co-factor. T and DHT were extracted by a newly developed DLLME technique and the compounds were then silylated and quantified by GC-MS. Td3 and DHT13C3

were used as internal standards. The proposed method showed good linearity (with correlation coefficients over 0.9987 for T and 0.09954 for DHT), good recoveries (higher than 80%), and good intra- and inter-day precision (below 13%, 3 levels, $n = 6$). The detection limits for T and DHT were 1 nM and the limits of quantification were 5 nM. The new GC-MS method was sensitive and precise for the simultaneous identification and quantification of T and DHT, allowing the evaluation of 5α-reductase activity, being a good alternative to the already described methods, which use radioactive compounds and TLC methodology.

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P04-92
Characterization of endothelial progenitor cells cultured from acute coronary syndrome patients

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Objective: Endothelial Progenitor Cells (EPC) are involved in integrity maintenance of the endothelium. EPC are also implicated in endothelium regeneration after infarction. We evaluated the functional capacity of EPC from acute coronary syndrome (ACS) patients to restore vascular lesions, in terms of adhesion, proliferation, growth curve and vasculogenesis.

Methods: 25 ml of blood were collected from ten patients with ACS and from ten healthy donnors. Mononuclear cells were plated for 30 days or until first EPC colonies appeared. Adhesion was evaluated by counting adhered EPC 30 min after seeding. Proliferation was assessed by flow cytometry and growth curve were performed from 1 to 6 days after seeding. Vasculogenesis was estimated by EPC ability to form tube-like structures on Matrigel. Human Umbilical Vein Endothelial Cells (HUVEC) were used as an endothelial cell model.

Results: EPC from controls and ACS patients exhibited measurable functional capacity after 30 days in culture. There were not differences in the adhesion properties of EPC between ACS, controls and HUVEC. However, the proliferative capacity of EPC was 37% higher in EPC in ACS than in controls and HUVEC ($p < 0.01$). EPC from ACS and control showed similar growth curves, whereas HUVEC grew faster from day 3 of culture ($p < 0.01$ versus control and ACS). Vasculogenesis was reduced in ACS when compared to controls and HUVEC ($p < 0.05$).

Conclusions: Although EPC from ACS patients proliferate faster than controls, they would be less capable of restoring the vascular wound. More detailed study of these functional parameters will lead to the development of new therapeutics in clinical treatments. (PRI-AIBDE-2011-0855), ISCIII (FIS PI08/0634, FIS 10/00518, RED HERACLES RD06/0009), C. Educación, GV (ACOMP/2012/218), Spain.

P04-93
Selenite activates ATM-dependent DNA repair pathway in osteosarcoma cells with chronic mitochondrial stress

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Mitochondrial chronic stress that originates from defective mitochondria alters cell physiology by affecting mitochondrial membrane potential, ATP, ROS and Ca^{2+} level, activity of respiratory chain complexes and organisation of mitochondria. In this study, we investigate effect of selenite on DNA repairing system in human osteosarcoma cells with two types of chronic mitochondrial stress determined by mutation in ATP synthase (T8993G mutation in mitochondrial genome, linked to a severe disease Neuropathy, Ataxia and Retinitis Pigmentosa) and by removal of mtDNA (Rho0). We found enhanced formation of DNA double strand breaks as measured by the level of histone γH2AX and active form of ATM kinase. Selenite did not reduce basal levels of oxidative modifications but increased activity of ATM kinase, ATM-mediated phosphorylation of p53 at *Ser* 15 and BRCA1 level (BRCA1 enables p53 phosphorylation at *Ser* 15 in a dose-dependent manner) in NARP cybrids and WT control cells. Selenite supplementation leads to the activation of ATM kinase and ATM-dependent DNA repair pathway not associated with an increase of DNA damage. These results suggest that selenite might keep ATM-dependent DNA repairing systems at the stand-by, and, thus, make cells more resistant to exogenous DNA damaging agents.

P04-94
Purification of glucose-6-phosphate dehydrogenase from acute myeloid leukemia patients leucocytes

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Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal leucocytes. Normal bone marrow produces erythrocytes, leucocytes and platelets. However in acute myeloid leukemia characterized with suppression of normal blood cell production and results with anaemia, leucopenia, and thrombocytopenia. One of the most important product of the pentose phosphate pathway is synthesis of ribose-5-phosphate, which is vital component of the DNA molecule. Glucose-6-phosphate dehydrogenase (G6PD) (D-glucose-6-phosphate: $NADP^+$ oxidoreductase, EC 1.1.1.49) is the first and the rate-limiting enzyme of this pathway. G6PD is a critical metabolic enzyme which affects many physiological processes by controlling the pathway and reducing $NADP^+$ to $NADPH + H^+$. Leucocytes were obtained from blood bank which was taken from the AML patients with therapeutic apheresis then refrigerated at -85°C. In this study we will describe a simple and a rapid purification method for the purification of G6PD from AML patients' leucocytes. All the procedures were carried out at +4°C. The leucocytes were homogenized after washing with 10 mM Tris/HCI buffer, pH 7.6, containing 1 mM 2-mercaptoethanol (2-ME) (buffer A). The purification consisted of 2′,5′-ADP-Sepharose 4B affinity and DEAE ion exchange

chromatography in procedure which took two working days. The enzyme was obtained with a yield of 30.8%. The overall purification was about 5882 fold. The effect of temperature and optimum pH of the enzyme activity was also examined. And we observed the pH curve has more than one maximum value (mainly at pH 8.0 and pH 9.5). This type of curve may be seen for diprotic systems and indicate that the active site of the enzyme may contain several ionizable groups.

P04-95
Variations in salivary function in a rodent model of pre-diabetes

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Diabetes is a widespread disease representing an enormous part of the total health costs. An early diagnostic could be of extremely importance both for the understanding and prevention of this pathology. Saliva is a fluid with increasing interest as a source of biomarkers for disease diagnostic and saliva protein composition changes have already been reported for diabetic individuals. However, the studies were performed after the onset of the disease and it is unknown if salivary changes are present in the early stages of development of the disease or a characteristic of overt diabetes. Wistar rats have been selected for their glucose intolerance (GIR). GIR females were compared with Wistar females with normal glucose tolerance (control) for changes in saliva protein composition and salivary gland histology. Fasting glycemias were observed to be normal (<95 mg/dl) in GIR animals, indicating an absence of a diabetic state. However they presented an abnormal increase in glycemia after a glucose bolus. For salivary parameters a marked increase in total protein concentration and alpha-amylase activity occurred in GIR animals, comparatively to controls. After separation of salivary proteins by SDS PAGE differences between the experimental groups for some protein bands, with apparent molecular masses ranging from 20 to 55 kDa were observed. Different expression of alpha-amylase at salivary gland duct level is also apparent for pre-diabetic animals. Although preliminary, these results suggest changes in saliva occurring before the onset of diabetes, reinforcing the interest of further investigation of saliva composition for the diagnostic of pre-diabetic condition, ultimately allowing an early intervention and eventually the prevention of disease development.

P04-96
Designing targeted drug delivery systems by targeting different cell surface receptors for thyroid cancer treatment

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Introduction: Nanotechnology has lead to an improvement in the diagnostics techniques and treatment of several diseases, using nanoparticles which transport the therapeutic agent specifically to the pathological area. Among many possible applications, tumors have been the most often investigated. Thyroid cancer is the most frequent endocrine neoplasm, and although differentiated cancers have in general a very good outcome, undifferentiated tumors, as in the case of anaplastic thyroid cancer, are more aggressive and do not respond to treatment. Our long term goal is to develop a targeted drug delivery system for thyroid cancer treatment, and for this purpose, we have first focused our work in the evaluation in cell cultures of polymer nanocarriers directed to potential targets on the surface of thyroid cells by fluorescence microscopy.

Experimental Methods: Model polymer nanocarriers (NCs) were prepared by coating green fluorescent polystyrene spheres (100 nm) by surface adsorption with different antibodies against the thyrotropin receptor (TSHR), and the epidermal growth factor receptor (EGFR), which is known to be overexpressed in a number of cancers. Binding and internalization of anti-TSHR or anti-EGFR NCs were tested by fluorescence microscopy in human thyroid follicular control cells (Nthy-ori 3-1), cells derived from a follicular carcinoma (FTC-133), and an anaplastic thyroid cancer cell line (8505C).

Results and discussion: All tested antibodies directed to TSHR and EGFR recognized thyroid cells as a monomolecular antibody. However, when coupled to nano-sized carries systems, only anti-EGFR nanocarriers bound efficiently to thyroid cells, although with different binding patterns, depending on the cell line. Furthermore, anti-EGFR NCs were internalized by thyroid cells in culture, mainly in the case of anaplastic thyroid cancer cell line. Our results provide an avenue to explore anti-EGFR NCs as transporters to specifically deliver therapeutic agents to thyroid cancer cells.

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P04-97
Localization of pyruvate kinase M2 (PKM2) expression in non-small cell lung cancer

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Lung cancer is the most commonly diagnosed cancer in the world and the first cause of death due to neoplastic disease. Despite advances in diagnosis and treatment, prognosis still remeins unfavourable.

Pyruvate kinase isozyme type M2 (PKM2), the enzyme catalyzing the rate-limiting final step of glycolysis, is consistently altered during tumorigenesis and is highly upregulated in tumors. Moreover, PKM2 promotes the Warburg effect. The aim of our study was to investigate the expression of PKM2 in non-small cell lung cancer (NSCLC) and in cancer associated fibroblasts (CAFs), which are known to support tumor growth and may play a key role in the acquisition of drug resistance in tumor cells.

The studies were conducted on 154 archival paraffin blocks of NSCLC from patients treated in the Lower Silesia Centre of Pulmonary Diseases in Wroclaw. Immunohistochemical reactions with PKM2 antibody were performed on paraffin sections using DAKO AutostainerLink 48. Expression of PKM2 was evaluated using the semi-quantitative immunoreactive (IRS) scale of Remmele and Stegner.

PKM2 was expressed significantly higher in NSCLC as compared to tissues of adjacent non-malignant lung tissues (NMLT). In NSCLC, PKM2 was expessed in cancer cells, as well as CAFs and tumor associated macrophages (TAMs). PKM2 expression in

cancer cells was higher as compared to CAFs and differed among histological subtypes of NSCLC.

Our results suggest that pyruvate kinase isozyme M2 expression may be involved in pathogenesis and progression of NSCLC, due to its higher expression in cancer cells and CAFs of this malignancy.

P04-98
CD26 and calprotectin as potential markers to design a panel for the detection of non-small cell lung cancer

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Lung cancer represents one of the most lethal neoplasias, making an early intervention crucial to guarantee better survival. However, no single marker can be expected to accurately define the presence of lung cancer. Potential markers that individually offer relevant diagnostic accuracy should be considered for inclusion in a panel to complement each other. In this work, levels of VEGF, an angiogenesis inducing factor; the soluble form of CD26, a membrane glycoprotein involved in cell cycle regulation and apoptosis, and calprotectin, an inflammation related molecule, were assayed by ELISA in serum from non-small cell lung cancer (NSCLC) patients, benign pathologies and healthy controls. Discrimination of NSCLC from other conditions was assessed by ROC curves and multivariate logistic regression to check if combination improved the AUC value. Reasonably, a graduation in marker levels was obtained through the different conditions studied. CD26 mean levels were: 396.9 ng/ml in NSCLC, 466.7 ng/ml in benign conditions and 527.6 ng/ml in healthy controls. For VEGF, levels were 694.2 pg/ml in NSCLC, 595.5 pg/ml in benign conditions and 518.1 pg/ml in healthy controls. For calprotectin levels in NSCLC were 237.2 ng/ml, in benign pathologies 193.5 ng/ml, and 126 ng/ml in healthy controls. The AUCs for the discrimination of NSCLC versus healthy controls resulted of 0.711 for CD26, 0.613 for VEGF and 0.825 for calprotectin. On multivariate analysis, VEGF remained not a significant predictor whilst conjunction of CD26 and calprotectin increased the AUC to 0.84. Combination of CD26 and calprotectin achieves acceptable distinction of NSCLC from healthy subjects. It is also worthy to analyse whether this combination could discern NSCLC from benign pathologies. Funding: Project PS09-00405 and Research Intensification activity from FIS, Xunta de Galicia and FEDER founding (CN 2011/024). Sonia Blanco: FPU.

P04-99
Value of serum epidermal growth factor receptor in the selection of patients with non-small-cell lung cancer to be treated with the tyrosine kinase inhibitor erlotinib

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Mutational analysis of epidermal growth factor receptor (EGFR) allows selection of patients with non-small cell lung cancer (NSCLC) with higher probability of a better response to EGFR tyrosine kinase inhibitors (EGFR-TKIs). Dissapointingly, the test is complex and requires tumour tissue. This study aimed to determine the value of pre-treatment serum EGFR on the prediction of response to anti-EGFR therapy. Serum samples from 44 patients diagnosed with NSCLC were collected between July 2009 and July 2011 before starting erlotinib treatment. Serum levels of sEGFR were quantified by an ELISA test (R & D Systems). Clinical data of patients were obtained from medical records. Overall survival (OS) and progression-free survival (PFS) were defined from the first day of treatment with the sEGFR-TKI to the date of death or progression. Survival curves were estimated using the Kaplan-Meier method and differences in survival between subgroups of patients were determined using the log rank test. For statistical analysis SPSS software (version 15.0) was used. Patients with sEGFR serum levels < 56.87 ng/ml before starting erlotinib treatment have a median OS of 4.2 months (95% CI, 0.6–7.8), which resulted significantly different from patients with serum levels > 56.87 ng/ml presenting an OS of 9.5 months (95% CI, 5.3–13.6), $p = 0.016$. In terms of PFS, serum levels < 56.87 ng/ml have a median PFS of 2.4 months (95% CI, 1.9–2.9), while patients with levels > 56.87 ng/ml have a PFS of 3.2 months (95% CI, 0.6–5.8), $p = 0.051$. Results are comparable to those obtained from the mutational study made on tumours of the same patients.

In conclusion, baseline sEGFR levels in NSCLC patients receiving sEGFR-TKIs correlated significantly with survival. The potential prognostic value of this serum marker could contribute to an adequate patient selection for expensive EGFR-TKI treatment.

P04-100
The influence of 'Hepafisan' capsules upon the processes of free radical oxidation under conditions of chronic hepatitis

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Activation of free radical oxidation (FRO) processes in case of liver pathology results in destruction of the cellular membranes, and later to death of the cells. The search of new medications able to regulate FRO processes with liver pathology is rather topical nowadays. The objective of the study was to examine 'Hepafisan' capsules effect upon the activity of FRO processes under conditions of chronic hepatitis caused in rats by tetrachlormethane and ethanol. 'Hepafisan' capsules (54 and 108 mg/kg) and 'Hepabene' capsules (88 mg/kg), as a referent medication, were administered orally.

The content of the products characterizing lipid peroxidation (LPO) condition was studied in the liver homogenate – thiobarbituric acid (TBA)-reagents, diene conjugates (DC), reduced gluta-

thione (RG), one of the component of the antioxidant system (AOS), as well as the content of cholesterol and general protein in the blood serum. Administration of 'Hepafisan' capsules resulted in the inhibition of free radical pathology development: DC content was three times lower with the dose of 54 mg/kg and two times lower with the dose of 108 mg/kg as compared with the indices of untreated animals. But reliable increase of RG level in 2.7 times was observed only in the dose of 108 mg/kg, which is indicative of the ability of 'Hepafisan' capsules to inhibit LPO processes on the early stage at the expense of AOS activation. This 'Hepafisan' capsules action is directed on homeostasis renewal in the liver, increased resistance of the organ to the influence of pathogenic factors, normalization of functional activity and stimulation of reparative-regenerative processes, which is indicative of increased survival ability of the animals to 88% (56% in the untreated animals with hepatitis), 1.5 times decreased mass liver coefficient and 2.3 times decreased cholesterol level, 1.5 times increased level of general protein as compared with the indices of the untreated animals with chronic hepatitis.

Under the influence of 'Hepabene' capsules the content of DC was two times lower, RG level was 1.7 times higher in the liver of animals with toxic hepatitis as compared with the indices of untreated animals. The results of the research detected dose-depended antioxidant activity of 'Hepafisan' capsules, which is higher than the activity of the referent medication. 'Hepafisan' capsules can be considered highly effective hepatoprotector with antioxidant mechanism of action preventing lesion of the cellular membranes and renewing the function of damaged hepatocytes in case of liver pathology by means of inhibition of FRO activation.

P04-101
Human epididymis protein 4 (HE4) as a new tumour marker for diagnosis of ovarian carcinoma

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Ovarian cancer is the fifth most common cancer in women, usually diagnosed at advanced stages due to low sensitivity and specificity of diagnostic approaches. CA125 is elevated 80–90% of stages III–IV patients but only in 50% of women with early stage ovarian cancer. Also, CA125 can be elevated in several benign conditions such as endometriosis, pregnancy, hemorrhagic cyst and pelvic inflammatory disease. Because of this, new tumour markers to detect early-stage ovarian cancer is necessary for asymptomatic patients, especially in particular of high-risk categories. HE4 (Human Epididymal Protein 4) is suggested to be a useful biomarker for the detection of ovarian cancer as it is expressed minimally in normal ovarian tissue. The purpose of this study is to evaluate the diagnostic and prognostic impact of serum human epididymis protein 4 (sHE4) in patients both in benign and gynaecological malignancies and to compare with CA125. HE4 and CA125 serum levels were determined in 50 healthy women (30 premenopausal and 20 menopausal), 45 patients with benign gynaecological diseases (endometriosis, myomas, ovarian cysts) 23 endometrial cancer and 53 ovarian cancers. Serum HE4 and CA125 concentrations were measured by quantitative chemiluminescent microparticle immunoassays (CMIA) on the automated Architect instrument (Abbott Diagnostics Division). Cut-off values was 35 U/ml for CA 125 and 150 pmol/l for HE4, respectively. In our study, sHE4 concentrations were found significantly higher in ovarian and endometrial cancer patients compared with benign conditions. Among patients with cancer, HE4 had significantly higher concentrations in ovarian cancer than in endometrial cancer, setting the specificity at 96%, the sensitivitiy at 65% for HE4 in ovarian cancer patients. Both HE4 and CA 125 were related to tumour stage and the use of both tumour markers improved the sensitivity obtained with only one tumour marker in all the stages. False positive results in patients with benign diseases were found in 4.1% and 28% for HE4 and CA 125, respectively. Our data indicate that HE4 is more sensitive and specific than CA125 in distinguishing early ovarian cancer patients from benign conditions.

P04-102
Crystallographic investigations of the complexes of plasmepsins with peptidic inhibitors

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Crystal structures of inhibitor complexes of two vacuolar pepsin-like proteases responsible for hemoglobin degradation by *Plasmodium falciparum,* plasmepsin I (PM-I) and histoaspartic protease (HAP), have been determined at medium resolution. Both proteins were cocrystallized with KNI-10006, a peptidic inhibitor containing an allophenylnorstatine-dimethylthioproline insert, and complexes of HAP with pepstatin and KNI-10395 were also obtained. Whereas the binding mode of pepstatin to HAP is rather similar to that found in its complexes with a variety of aspartic proteases, KNI-10006 binds to both PM-I and HAP in non-standard ways. This inhibitor binds near the catalytic center of PM-I but its direction is opposite to the putative direction of the substrate, likely due to the need to preserve the stereochemistry of interactions of its central hydroxyl group of with the catalytic aspartates. The binding mode of KNI-10006 to HAP is drastically different from that of pepstatin A. In the HAP complex, the hydroxyl group in the central part of the inhibitor points away from the catalytic residues, in contrast to its orientation in the structures of PM-I, PM-IV, or HIV-1 PR, where it is positioned between the active-site aspartates. The predominant interactions of KNI-10006 are with the flap, but not with a number of hydrophobic residues conserved in plasmepsins and in other pepsin-like enzymes. KNI-10395 is found in an unprecedented conformation resembling the letter 'U', stabilized by two intramolecular hydrogen bonds. The novel features of the inhibitor complexes of PM-I and HAP will facilitate design of specific compounds that might provide leads for the development of antimalarial drugs.

P04-103
Effect of triterpene saponosides on human prostate and normal cells *in vitro*

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Despite of fact that process of carcinogenesis is well known and its mechanism is extensively studied there is still no effective cure for cancer disease. Traditional cancer chemotherapy frequently uses cytotoxic agents to destroy cancer cells at the expense of normal host tissue. Very important is search new chemotherapeutics agents that are effective will be incorporated with an outbreak of disease with minimal effect on healthy tissue.

Triterpenoid saponosides are secondary metabolites derived from higher plants. Biological activities of saponins are well known and they include: antimicrobial, antiinflamatory, expectorant as well as anticancer action.

Published data suggest the correlation between chemical structure of saponosides and their biological activities, however the exact influence on cancer cells is yet unknown. Subtle changes in substituents in the main chain of the chemical structure of saponins determine different biological activity.

In this study we analyzed saponosides with different chemical structure and compared their impact on the cancer and normal cells (Du-145, PC3 human prostate cancer cell line with different metastasis potential, PNT2 normal prostate cell line). Analysis of cells vital function include proliferation, morphology, mechanism of cell death, migration and cytoskeleton organization and cell elasticity. Preliminary result of our study indicate that these saponosides have a high selectivity in their effect on the examined cells and what is more the effect of studied saponosides is more pronounced on cancer cells than effect of mitoxantrone- commonly used in cancer therapy drug, in contrast to normal cells. Our data suggest that subtle changes in the chemical structure of saponins have a significant impact under biological role.

P04-104
Quantitative phosphoproteomics dissects sensitization of EGF-resistant colon cancer cells to tyrosine kinase inhibitors through spliceosome modulation

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The identification of molecular biomarkers of response is a critical step towards optimization of efficacy in personalized cancer therapy. The precise understanding of cross-talks between drug-modulated and resistance conferring pathways is key to this process. Mutations within parallel or cross-sectioning signaling cascades confer resistance against Epidermal Growth Factor Receptor (EGFR) therapies. Dissecting inter-molecular interactions in these pathways is therefore essential to overcome resistance and optimize anti-EGFR treatments.

Herein we provide strong evidence that g-Tocotrienol (g-T), a minimally toxic derivative of vitamin E with potent signal-modulating properties, differentially modulates the efficacy of EGFR Tyrosine Kinase Inhibitors (TKI) in colon cancer models with diverse EGFR expression. A quantitative phosphoproteomics study in g-T-treated cells disclosed significant new hits within major spliceosome and nucleosome protein targets and differential expression of kinases associated with the EGFR cascade. Combined with TKI in TKI-non-responding cells, g-T elicited additional hits thus defining new intracellular targets. Bioluminescence/Fluorescence Resonance Energy Transfer (BRET/FRET) systems have enabled the monitoring of inter-molecular interactions between established and newly identified target proteins in live-cell settings. FRET signals induced by fluorescently labeled g-T suggested that EGFR may serve as a candidate target of g-T. These findings are further supported by docking experiments which reveal putative recognition of the active site of EGFR (wt) kinase domain by g-T. Interestingly, some predicted γ-T binding poses resemble closely the binding mode of TKI in the active site, exemplified by the creation of specific H-bonds to the kinase hinge region (residues M793, Q791), similar to the TKI.

Conclusively, our findings provide substantial evidence for the role of g-T in overcoming resistance to anti-EGFR therapies and are, therefore, expected to further enable patient stratification for combined therapies.

P05 – Biomembranes and Bioenergetics

P05-1
Reconstruction of electrogenic charge transfer reactions on the donor side of the Mn-reassembled apo-water oxidation complex of photosystem II

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Photosystem II (PS II) catalyses the light-driven oxidation of water, evolving one molecule of oxygen and four protons per two water molecules and reduction of plastoquinone to plastohydroquinone. Water oxidation and oxygen evolution are completely inhibited upon depletion of Mn ions from the water-oxidation complex (WOC) which is designated as apo-WOC-PS II particles. However, the WOC can be reversibly reassembled in the presence of the free inorganic ions (Mn^{2+}, Ca^{2+}, Cl^-) and PS II reaction centers which are capable to perform this complex process. This process which also occurs in the native assembly of the manganese cluster under various stresses is called photoactivation. The electrometrical technique was used to study electrogenic (vectorial) reactions on the donor side of photoactivated apo-WOC-PS II core complexes reconstituted into phospholipids vesicles. In dark-adapted samples, fast generation of the voltage transients due to electron transfer from the redox active tyrosine residue (YZ) to the primary plastoquinone acceptor (QA) induced by three successive laser flashes was followed by an additional electrogenic phases in the kinetics of the photoelectric responses with lifetimes of 40 μs, 220 μs and 5 ms, which were ascribed to S1-S2, S2-S3 and S4-S0 transitions, respectively. The phase S1-S2 is likely related to vectorial electron transfer from the Mn to the tyrosine radical YZ• while transitions S2-S3 and S4-S0 are due to transport of proton(s) in the opposite direction from the Mn complex or its immediate environment to the bulk water. The data obtained demonstrate the possibility of reconstruction of the apo-WOC-PS II functioning *in vitro*.

P05r-2
The role of positively charged amino acids in the C1B domain of PKCε and their electrostatic interactions with lipid membranes

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C1 domains are members of the Cys-rich domains superfamily, formed by 50–51 amino acids residues present in many types of proteins, as it is the case of the classical and novel Protein Kinases C (PKCs). Both types of PKCs, classical and novel isoenzymes, possess two C1 subdomains, C1A and C1B, although it is not totally clear why two modules are needed. C1 domains are known to interact with diacylglycerol and exogenous agents like phorbol esters.

In a previous report, we demonstrated the importance of not only diacylglycerol but also of anionic phospholipids, specifically 1-palmitoyl-2oleoyl-*sn*-glycero-3-phosphate (POPA) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), for the interaction of the C1 domain with lipid membranes, among which the C1Bε domain had the highest binding affinity than any other C1B domains of novel isoenzymes.

In this work we have studied the role of positively charged amino acids residues located on top of the C1Bε domain and their involvement in the interaction membrane-protein. Selected amino acids residues were replaced by Alanine and we characterized the effect of single, double and triple mutations.

To carry out this study, the different C1Bε mutants were expressed fused eCFP in HEK293 cells. Fluorescent constructs were obtained from cell lysates and binding to lipid vesicles (LUV) labelled with Oregon Green was monitored through FRET.

Furthermore, each mutant was expressed as well in RBL-2H3 cells and their subcellular localization studied after stimulation with different concentrations of 1,2-dioctanoyl-*sn*-glycerol and 1,2-dioctanoyl-*sn*-glycerol 3-phosphate (PtdOH).

Results show that binding is decreased by increasing of the number of residues mutated in the domain, and it be even abolished in the presence of diacylglycerol.

In conclusion, the electrostatic interactions derived of these positively amino acids residues is important to give place to membrane docking which is further stabilized by interaction with the diacylglycerol.

P05-3
Effects of diphenylene iodonium on hydrogen production and membrane ATPase activity in *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides MDC6521 (isolated from mineral springs in Armenia) is capable of growing and producing biohydrogen (H_2) with high rate from succinate in anaerobic conditions and nitrogen source limitation under illumination in 1500 lx, pH 7.0–7.2 (28–30°C). In the present work, ATPase activity and H_2 production by *R. sphaeroides* MDC6521, are studied, the effect of diphenylene iodonium (Ph_2I), inhibitor of hydrogenase, on these processes is shown.

The H_2 producing ability of bacteria was totally suppressed in the presence of Ph_2I. In order to examine the mediatory role of the F_0F_1-ATPase in H_2 production by *R. sphaeroides*, the effects of Ph_2I on ATPase activity was investigated. The membrane vesicles of *R. sphaeroides* demonstrated significant ATPase activity, determined by the liberation of inorganic phosphate in the reaction with ATP. The incubation of membrane vesicles in the presence of Ph_2I caused to marked inhibition in ATPase activity. The concentration dependent Ph_2I effect was observed: ATPase activity was not affected at the concentrations of Ph_2I up to 10 μM, and the inhibition of ATPase activity was enhanced with increase of Ph_2I concentration. Incubation of membrane vesicles in the presence of 40 μM Ph_2I led to significant (~2 fold) inhibition in ATPase activity.

The results obtained point out the role of hydrogenase in H_2 production by *R. sphaeroides* under nitrogen limitation conditions. Moreover, relationship of hydrogenase with the F_0F_1-ATPase is suggested.

P05-4
Impact of docosahexaenoic fatty acid (w-3) on the formation of liquid-ordered domains

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Docosahexaenoic acid (DHA), belonging to the family of w-3 fatty acids, is the most abundant polyunsaturated fatty acid in the human central nervous system. DHA has an essential role in brain function. It is supposed that one of the mechanisms of its action is to affect the organization of biological membranes. In order to better understand DHA phase behavior in membrane systems, phosphatidylcholine/sphingomyelin/cholesterol (PC/SM/CHOL) ternary mixtures were investigated using differential scanning calorimetry and fluorescence microscopy. Two phosphatidylcholine species were used for comparative study: palmitoyl-oleoyl phosphatidylcholine (POPC) and palmitoyl-docosahexaenoyl (w-3) phosphatidylcholine (PDPC) differing in the number of double bonds at the *sn*-2 position (1 for POPC and 6 for PDPC). The PDPC-containing mixtures showed a lower cooperativity of the transition than the POPC-mixtures demonstrating the increased heterogenity of the membrane bilayer. Moreover, PDPC-containing mixtures induced the formation of a more ordered phase with a higher Tm and delta H than the one formed in the presence of POPC. Fluorescence microscopy observations showed that DHA induced formation of micron-scale liquid ordered domains (model of cellular '*rafts*') at physiological temperature and higher ones in difference with the monounsaturated oleic acid. The present results suggest that DHA could participate indirectly in the process of *raft* domain formation by reducing the miscibility of SM and CHOL for liquid disordered phase, thus more of these molecules would be engaged in the formation of liquid-ordered phase.

P05r-5
Inhibitory effects of antioxidants on neonatal rat cardiac myocyte cellular death induced by 4-hydroxynonenal

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Lipid peroxidation, induced by oxidative stress, is associated with degenerative processes. HNE, the major product from lipid peroxidation, has a causal role in cytotoxicity and it has been related with the pathophysiology of cardiovascular diseases, such as myocardial infarction (MI) and Heart Failure (HF)[1]. The aim of this study is to analyze the action of a wide range of antioxidant products, in order to protect against HNE damage in neonatal rat cardiomyocytes (NRC). Our results showed a toxic effect of HNE in NRC, being HNE 4.5 μM the LC50, which is categorized in the physiopathology level of HNE under oxidative stress. The cell death is mediated by apoptosis, with Ca^{2+} overload, deep mitochondrial alterations (ROS overproduction and loss of $\Delta\Psi m$) and decrease of ATP and GSH levels. Cell death induced by HNE is prevented by the antioxidant trolox; the β-blocker carvedilol; the thiol compound N-Acetyl-Cysteine (NAC) and β-mercapto-propionyl-glycine (MPG); as well as a natural compound whose antioxidant power has been described *in vitro*, it is the essential oil of *Thymbra capitata*. It is interesting to highlight: both, NAC and MPG, which are able to restore the GSH pool; as well as the *Thymbra capitata* essential oil, with high level of carvacrol, which results to be very potent, being enough 0.002% of essential oil to prevent the cell death as well as the ROS generation and loss of $\Delta\Psi m$.

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P05-6
Bioengineering of the ATP synthase: customized ion-to-ATP ratios and ion specificity

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F_1F_o-ATP synthase membrane rotor consist of a ring of c-subunits (c-ring) where each subunit provide one ion binding site and contribute to the ion specificity of the enzyme. In our study we addressed the question how the ATP synthase 'ion-to-ATP ratio' could be manipulated. We investigated the importance of c/c-subunit contacts by site-directed mutagenesis of a conserved stretch of glycines in a c_{11} ring from *Ilyobacter tartaricus*. Structural and biochemical studies show a direct influence of mutations on the c-subunit stoichiometry, revealing c_{10-16} rings. Protein interaction studies demonstrate that the assembly of the F_1F_o rotor complex is independent of the c-ring size. Furthermore, the mutant ATP synthase with the larger (c_{12}) than wild-type (c_{11}) ring indeed produces ATP at the lower membrane potential threshold – that is, at higher ion-to-ATP ratio.

Further, we addressed the question how the Na^+ versus H^+ ion-binding selectivity of the F_o rotor could be manipulated. We produced a set of *I. tartaricus* c-ring mutants with substitutions of the critical residues at the ion-binding site. To probe the differential ion-binding properties of these mutants we measured the ATP synthesis rates and extent of DCCD labelling in response to different pH and salt concentrations. Our results suggest that the inherent Na^+ selectivity of c_{11} ring can be adjusted by specific point mutations in the c-subunit in the way, that the selectivity of the binding site, under physiological conditions, is drastically enhanced for H^+ against Na^+.

Finally, the revealed in this work structural and functional compliance in the architecture of the rotor-ring provide us with the exciting possibility to design and engineer functional ATP synthases with customized ion-to-ATP ratios and ion specificity.

P05-7
The PI(4,5)P_2–binding site in C2 domains: a conserved motif with subtle variations that modulate their membrane interactions

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C2 modules are most commonly found in proteins involved in lipid modifications, signal transduction, and membrane trafficking. Many of these C2 domains act as cellular Ca^{2+} sensors.

Calcium ions bind in a cup-shaped depression at one tip of the β-sandwich with incomplete coordination spheres that can be occupied by neutral and anionic phospholipids, enabling the C2 domain to dock at the membrane. Previous studies in our laboratory have shown that the C2 domain of PKCα can interact with both phosphatidylserine and $PI(4,5)P_2$simultaneously, revealing an specific $PI(4,5)P_2$-binding site located in a polybasic region at the β3-β4 strands.

Here, we characterized the function of this polybasic region in the C2A and C2B domains of synaptotagmin 1 and rabphilin 3A. Crystallographic studies confirm that $PI(4,5)P_2$also interacts with residues located at the β3-β4 strands of the C2A domain of rabphilin 3A. ITC and FRET measurements combined with site-directed mutagenesis revealed that all these C2 domains share a collection of conserved amino acids that allow them to interact with $PI(4,5)P_2$. However, there are many residues located in the vicinity of this motif that provide every domain with the ability to interact with other different phosphoinositides. Taken together, these results enable us to propose a molecular mechanism to explain the specificity of each particular C2 domain-membrane interaction.

P05r-8
Cellular models for the study of pulmonary surfactant biogenesis and functional mechanisms

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Pulmonary surfactant is a lipid-protein complex that reduces surface tension at the air-liquid interface in the alveoli preventing their collapse at end-expiration. Lung surfactant is synthesized and secreted to the respiratory surface by pneumocyte type II or alveolar type II cells (AT II cells). Prior to exocytosis, surfactant membranes are stored in special lysosomal-related organelles called lamellar bodies (LBs). Material secreted from LBPs maintains a densely packed structure in the form of lamellar body-like particles (LBPs), which disintegrate in a cooperative manner after reaching the air-liquid interface to generate the surface active film.

To study in depth the biosynthetic pathways involved in surfactant biogenesis as well as some key events in the mechanisms of its surface action, we are taking advantage of the use of primary cell cultures.

On the one hand, differentiation from placenta-derived mesenchymal stem cells to cells with phenotypical features similar to pneumocytes (Pneumocyte-like Cells, PLCs) has been developed. PLCs are able to synthesize surfactant proteins and contain intracellular organelles resembling LBs. PLCs are also sensitive to secretagoges and upon stimulation, they exocytate a lipid material resembling surfactant membranes.

On the other hand, exocytosis assays in rat AT II cell primary cultures have been intensely optimized, becoming this cultures in reliable sources to obtain secreted surfactant material in form of LBPs. These LBPs have been functional and structural characterized from several approaches, revealing significant evidences in structural complexity and surface active efficiency.

A proper cellular models developing could be a very valuable approach to shed light on many processes related to pulmonary surfactant which are still unclear.

P05-9
Mitochondrial porin: a new subunit of the TIM22 protein translocase

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Mitochondrial biogenesis entirely depends on the import of approximately 1000 different proteins, which are transported from the cytoplasm to one of four distinct compartments enclosed within the two membranes of mitochondria. The intricacy of the protein import processes is reflected in the recent description of at least five multiprotein complexes, or translocases, whose molecular mechanisms are largely unknown. Moreover, different approaches are enabling us to report unprecedented and unexpected interactions between translocases and other mitochondrial proteins. We present new evidence for a structural interaction between TIM22 translocase, responsible for the insertion of proteins into the inner membrane, and the mitochondrial porin VDAC (Voltage Dependent Anion Channel) whose most prominent function is the transport of low molecular weight metabolites through the outer membrane. We have used mitochondria isolated from 12 *S. cerevisiae* strains with mutations in Tim22p, Tim18p and Tim54p (membrane components of TIM22) as well as VDAC1 (that forms the channel) and VDAC2 (an isoform with an unknown function yet). The results obtained using blue native polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis, as well as co-immunoprecipitation and real-time PCR experiments, suggest that there is a specific structural association between the major isoform of the mitochondrial porin (VDAC1) and the TIM22 translocase. In addition, the integrity of the TIM22 complex not only depends on the simultaneous presence of its three membrane components, but it also relies on the presence of VDAC2.

Taken together, these results point to VDAC as a new subunit of the TIM22 complex and for the first time, allow us to assign a possible role to VDAC2 through its involvement in the biogenesis of the mitochondrial protein import translocase TIM22.

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P05-10
In situ synthesis of fluorescent membrane lipids (ceramides) using click chemistry

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Abstract Ceramide analogues containing azide groups either in the polar head or in the hydrocarbon chains are non-fluorescent. When incorporated into phospholipid bilayers, they can react *in situ* with a non-fluorescent 1,8-naphthalimide using click chemis-

try giving rise to fluorescent ceramide derivatives emitting at ~440 nm. When incorporated into giant unilamellar vesicles, two-photon excitation at 760 nm allows visualization of the ceramide-containing bilayers. This kind of method may be of general applicability in the study of model and cell membranes.

P05-11
Effect of cholesterol and palmitoylation on the structure, orientation and lipid-protein interactions of pulmonary surfactant protein SP-C

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Pulmonary surfactant is a lipid/protein complex covering the inner surface of the lungs, where it is responsible for reducing the surface tension of the respiratory air-liquid interface and so to prevent alveolar collapse at the end of expiration, favoring oxygen diffusion. The composition of pulmonary surfactant varies slightly between species but in general it consists of 90% lipids by weight and 8–10% proteins, including the specific surfactant proteins SP-A, SP-B, SP-C and SP-D. The main feature of protein SP-C (35 amino acids, 3.7 kDa) is its extreme hidrophobicity. Structurally it consists of an N-terminal segment and a α-helical transmembrane segment. The positions 5 and 6 of SP-C sequence are in most species occupied by two cysteines that are palmitoylated. It has been proposed that SP-C interacts with cholesterol-rich phases of surfactant membranes and films, contributing to modulate the interfacial behavior of surfactant in the alveoli (1,2), but evidence remains uncertain. Our aim in this work was to elucidate how palmitoylation influences the interaction of SP-C with lipids in different surfactant-mimetic systems in the presence or absence of cholesterol. To this end, we have compared the secondary structure, tilting and lipid-protein interactions of native palmitoylated SP-C purified from porcine lungs with the behavior of non-palmitoylated recombinant versions of SP-C produced in bacteria, by means of ATR-FTIR. This technique is sensitive to the orientation, structure and interactions of SP-C in membranes (3), which can be correlated with the contribution of SP-C to stabilize compressed cholesterol-containing surfactant films.

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P05-12
Tollip – a new player in multivesicular body biogenesis

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The multivesicular body (MVB) constitutes a key hub for membrane protein trafficking wherein the endocytic, recycling and degradation pathways intersect. Sorting of cargo to and from this subcellular compartment employs ESCRT complexes. Importantly, MVB can be formed without the participation of known ESCRT proteins, suggesting the involvement of previously uncharacterized players in MVB biogenesis and associated vesicular dynamics.

To identify novel proteins involved in MVB formation we performed a genome-wide RNAi screen revealing five proteins that could control this process (1).

Among these candidates, Tollip differentially controls cell surface receptor distribution with respect to ubiquitylation and its depletion results in abnormally enlarged endosomal structures. This enlarged MVB compartment is further characterized by decreased amount of intralumenal vesicles and higher lipid content relative to the control. Re-expression of siRNA-resistant Tollip rescues these perturbations in MVBs dynamics. Ous data suggest that Tollip controls transport of endocytosed receptors to, within, and from MVB via either direct or indirect interaction with lipids.

Tollip interacts with members of the Tom1 family to bind ubiquitylated proteins and clathrin. These properties resemble the ones of Hrs and STAM – components of a canonical ESCRT-0. Importantly, because Tollip does not interact with Hrs and localizes to a distinct population of endosomes, we propose the existence of a novel ESCRT complex formed by Tollip that contains Tom1 family member(s), which may facilitate the recruitment of ubiquitin-modified proteins to MVB.

Our ongoing work implicates Tollip involved in spatiotemporal regulation of vesicular dynamics and MVB integrity.

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P05m-13
A soluble factor in plasma of caloric restricted rats modifies insulin secretion in a mitofusin-2 dependent manner

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One of the well described effects of calorie restriction (CR) is the reduction in blood insulin levels and prevention from Diabetes 2 development. Changes in serum hormones and cytokines are enough to reproduce some of the CR effects *in vitro*. CR sera increase mitochondrial biogenesis and reserve respiratory capacity in neurons. Insulin-producing cells rely mostly on mitochondrial respiration for the ATP synthesis. We hypothesized that serum changes promoted by CR could modulate insulin release due to enhanced mitochondrial mass. We addressed this hypothesis by culturing INS1 cells with media in which serum from rats fed *al libitum* (AL) or submitted to CR were used instead of fetal bovine serum. CR sera increased mitochondrial mass and mitofusin-2 (Mfn2) expression, associated with highly branched mitochondria levels. On the other hand, glucolipotoxicity (GLT) (an *in vitro* model of Diabetes 2) lead to mitochondrial mass loss, associated to mitochondrial fragmentation. Basal and glucose-stimulated respiration were significantly higher in cells cultured with CR sera, however these cells presented an attenuated fold in insulin secretion in response to glucose. This effect was reverted after Mfn2 knockdown (Mfn-2 KD). The oligomycin-resistant respiration was lower in Mfn-2 KD cells suggesting that Mfn-2 KD reduces the proton leak. Changing glucose levels from 2 to 12 mM promoted enhanced DCF (a non-selective reactive oxygen species probe) fluorescence, in a manner prevented by CR sera, probably because of the higher mitochondrial uncoupling. In Mfn-2 KD cells, DCF fluorescence was much higher in both AL and CR sera. Overall, our results indicate that serum changes promoted by CR increase mitochondrial mass and morphology through Mfn-2 overexpression. Mfn-2 overexpression increased uncoupled respiration and reduces oxidant release, which can be a mechanism of reduced insulin secretion after the treatment with CR sera. Mitochondrial morphology manipulation can be a tar-

get to prevent insulin deregulated production during the early phases of Diabetes.

P05-14
Role of membrane cholesterol in hydrophobic matching and the resulting redistribution of proteins and lipids

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One of the physical mechanisms leading to lateral self-organization of cell membranes is the hydrophobic mismatch between a lipid membrane and the transmembrane part of a membrane protein. Meanwhile, cholesterol is in many ways a unique molecule with regard to its capability to promote membrane order and control the physical properties of lipids around it. In this spirit, it is tempting to consider how cholesterol could contribute to hydrophobic mismatch. The topic is particularly exciting given that there is a gradient of cholesterol along the secretory pathway, implying that the changes in membrane properties due to varying concentration of cholesterol can be an important factor for the sorting of non-matched Golgi transmembrane proteins.

We have combined atomistic simulations with a major arsenal of experimental techniques to study the role of cholesterol in hydrophobic mismatch as well as its biological consequences. We have observed cholesterol to play a central role in controlling structural adaptations at the protein-lipid interface under mismatch. This is shown to result in a sorting potential that leads to selective segregation of proteins and lipids according to their hydrophobic length. The results allow us to provide a mechanistic framework for a better description of the organizing role of cholesterol in eukaryotic membranes.

P05-15
The role of SCaMC-3, the mitochondrial transporter of ATP-Mg/Pi expressed in liver and brain, in regulating OXPHOS

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Mitochondria isolated from hepatocytes treated with glucagon or calcium (Ca^{2+})-mobilising agents such as phenylephrine show an increase in their adenine nucleotide (AdN) content. It has been suggested that this net uptake of AdN by liver mitochondria is catalysed by the Ca^{2+}-dependent ATP-Mg/Pi carrier, but the extent to which this increase may regulate the effects of glucagon in liver cells remains unclear. Here, we have studied the role of SCaMC-3, the mitochondrial ATP-Mg/Pi carrier present in mouse liver, on mitochondrial function in response to calcium signals. Our results show that Ca^{2+} activation of SCaMC-3 induces uptake of ATP-Mg or ADP in isolated mitochondria, and that this increase in mitochondrial AdN content results in an increase in state 3 respiration.

This mechanism is shown to be physiologically relevant *in vivo*, as liver mitochondria lacking SCaMC-3 fail to increase their AdN content after glucagon treatment, and isolated hepatocytes from SCaMC-3 KO mice show reduced stimulation of respiration. Taken together, these results indicate that SCaMC-3 is a main target of glucagon in mouse liver mitochondria.

P05-16
Hydroxytyrosol prevents mitochondrial respiratory dysfunctions in serum-limited human fibroblasts

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The use of olive oil in the diet appears to be associated with a lower incidence of cardiovascular diseases and cancer in the Mediterranean population. Hydroxytyrosol (HT), the major o-diphenol present in extra-virgin olive oil, is considered to contribute significantly to the health benefit of olive oil. Studies on the action of HT have been essentially focused on its antioxidant value.

Recently stimulation by HT of the expression of the PGC-1a, master gene, TFAM, NRF1and NRF2 has been observed. Stimulation of this transcription factor cascade was shown to be accompanied by enhanced expression of subunit proteins of oxidative phosphorylation complexes. We have studied the effect of HT on the reduced level of expression and activity of mitochondrial respiratory system induced, in human fibroblasts cultures, by serum limitation. Low concentrations of HT (< 1 mM) prevent the decrease of the level of subunits and enzymatic activity of complex I, and reduce reactive oxygen species production caused by serum limitation. These effects appear to be associated with stimulation by HT of PGC-1a expression. These results substantiate a beneficial mitochondrial targeting action of HT.

Keywords: biogenesis, complex I, free radical, hydroxytyrosol, mitochondria.

P05-17
Lessons from reductionism on a membrane-active protein: minimal pore-forming versions of Bax

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Amphitropic proteins of the Bcl-2 family regulate apoptosis by controlling the permeability of the outer mitochondrial membrane. Although this appears to be due to Bax pores, their nature, mechanism and main properties remain elusive, due to the lack of structural information of the protein/membrane complexes. However, we have shown that the main-alpha structure of Bax permits the design and study of minimal active versions of this protein. Peptides encompassing the sequences of amphipathic helices from the central hairpin of Bax, Bid and Bcl-xL exhibit membrane binding and permeabilizing activities which resemble those of the respective complete proteins. The Bax peptide fragments are particularly potent for membrane poration. A detailed study of their action mechanism, including modeling and quantitative analysis of the kinetics of vesicle leakage, allows counting and sizing the pores and studying their formation, equilibration

and dynamics. This work establishes mechanistic links between the activity of pore-forming proteins and peptides. In parallel, it sets the basis for the design of a new family of potent drugs with potential antibiotic and antitumor activities.

P05-18
Dibenzofuran and mitochondria: interference with phosphorylation 'ANT' permeability transition

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Dioxins and furans are very toxic and exposure to these environmental pollutants, such as fuel constituents, is linked to several diseases. Dibenzofuran is listed as a pollutant of concern due to its persistence in the environment, bioaccumulation and toxicity to humans and the environment.

Mitochondrial function is very important in cellular homeostasis and keeping a proper energy supply for eukaryotic cells is essential in the fulfillment of the tissues energy-demand. The main objectives of this work concerned Dibenzofuran effects on mitochondrial function. We isolated mitochondria from rat liver and incubated them with Dibenzofuran to analyze the effects of this pollutant at the level of mitochondrial function.

The effects of Dibenzofuran exposure include a markedly increase in the lag phase that follows depolarization induced by ADP, indicating an effect in the phosphorylative system. Experiments performed using carboxyatractyloside (CAT) suggested an interaction of Dibenzofuran with the ANT carrier. Dibenzofuran exposure also produces an inhibition of mitochondrial permeability transition and an increase in calcium retention capacity, which may also be explained by a putative interaction of Dibenzofuran with ANT.

Clarifying the role of pollutants in some mechanisms of toxicity, such as unbalance of bioenergetics status and mitochondrial function, may help to explain the progressive and chronic evolution of diseases derived from exposure to environmental pollutants.

P05-19
Inhibition of the secretory pathway Ca^{2+}/Mn^{2+}-ATPase (SPCA) activity and Golgi fragmentation is caused by Mn^{2+} toxicity in cultured neurons and glia

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Manganese (Mn^{2+}) is a trace element essential for brain. However, Mn^{2+} excess can have a potent neurotoxicity effect. Since Mn^{2+} toxicity in brain is poorly understood, we have prepared mouse primary cultures of neurons and glia in order to address the cellular impact of high extracellular Mn^{2+} levels. Incubation with increasing concentrations of Mn^{2+} drastically reduced survival of both cell types, being glia more resistant as shown by MTT assays. Analysis of subcellular morphology by immunofluorescence revealed disruptions of the cytoskeletal scaffold, structural rearrangements of endoplasmic reticulum and fragmentation of the Golgi apparatus. Although no changes in mitochondrial morphology were observed, a mitochondrial dysfunction was pointed out by increased ROS production, related with an increase of apoptotic cells. Taking into account that the Golgi houses the Ca^{2+}/Mn^{2+}-ATPase of the secretory pathway (SPCA), which currently might represent the only known way for cellular Mn^{2+} detoxification, we analyzed the impact of Mn^{2+} overload on SPCA function. ATPase activity assays showed stimulation of the Mn^{2+}-dependent SPCA activity at concentrations below 0.01 mM free-Mn^{2+} and inhibition at higher concentrations. Besides, high levels of Mn^{2+} also inhibited the Ca^{2+}-dependent SPCA activity and Ca^{2+} could inhibit the Mn^{2+}-ATPase activity, suggesting a competition of both ions, Ca^{2+} and Mn^{2+}, for SPCA binding. These results showed that Mn^{2+} overload may affect Ca^{2+} and/or Mn^{2+} homeostasis via SPCA, affecting Golgi functions and overall cellular signaling. Additionally, we have explored the reversibility of Mn^{2+} effects in order to asses possible therapeutically approaches.

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P05-20
Modulation of pig brain plasma membrane Ca^{2+}-ATPase by amyloid β-peptide and calmodulin

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Synaptosomal plasma membrane Ca^{2+}-ATPases (PMCAs) are high-affinity Ca^{2+} transporters that efficiently extrude the excess of Ca^{2+} from the cell. Therefore, they are essential regulators of Ca^{2+} homeostasis and Ca^{2+} signaling events in the nervous system. The disruption of Ca^{2+} signaling has been proposed as a key factor for neuronal dysfunction in the Ca^{2+} hypothesis of Alzheimer's disease (AD). The presence of amyloid-β peptide in different states of aggregation and as senile plaques is a neurotoxic hallmark of this disease and it has been closely related to Ca^{2+} dysregulation. In relation to that we have recently revealed the impairment of synaptosomal PMCA in AD linked to an inhibitory effect of the neurotoxic amyloid-β peptide (Aβ). To deep inside into the mechanism of Aβ inhibition we hypothesize that molecules that regulate the PMCA function may also affect the toxic effect of Aβ on PMCA and hence on Ca^{2+} dysregulation. Here, we show that Aβ peptides bind to the calmodulin binding domain on PMCA. Besides, this inhibition is modulated by calmodulin, being blocked by concentrations of calmodulin above 5 nM. Immunological assays and measurements of intrinsic fluorescence point out an interaction of Aβ with both, PMCA and calmodulin. Thus, calmodulin could protect PMCA from its inhibition by Aβ by burying exposed sites on PMCA, making them inaccessible to Aβ, and also by direct binding to the peptide. Overall, we propose that calmodulin may be an important mediator of Ca^{2+} dysregulation in AD due to its interaction with both, Aβ and PMCA, and suggest a mechanism for the observed effects.

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P05-21
Specificity of quinone catalysis by CymA; a c-type cytochrome central to anaerobic metabolism in Shewanella oneidensis MR-1

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CymA is a membrane-bound tetraheme c-type cytochrome belonging to the NapC/NirT family in Shewanella oneidensis MR-1. CymA has been predicted to mediate almost all the anaerobic electron transfer reactions through the menaquinone pool to a diverse range of final electron acceptors. Here we have interrogated the catalytic properties of CymA using protein-film voltammetry and artificial supported lipid bilayers on SAM-modified electrodes. CymA is specific for menaquinone (MQ-7) conversion and cannot directly catalyse oxidoreduction of ubiquinone (UQ-10) or menadione (MQ-0). MQ-7 tightly binds to CymA, and in this state CymA is able to reduce both UQ-10 and MQ-0, suggesting MQ-7 is not only a substrate, but also a cofactor. Despite a role of MQ-7 oxidation *in vivo*, purified CymA is strongly biased towards MQ-7 reduction; no MQ-7 oxidation was observed. However, when CymA is bound to a binding partner coupled to a final electron acceptor, such as FCC3/fumerate, oxidative catalysis is observed. This leads to the hypothesis that the electron accepting/donating binding partner and/or other accessory proteins have significant roles in the functional mode of this enzyme.

P05-22
Mitochondrial proteome analysis reveals depression of Ndufs3 subunit and activity of complex I in diabetic rat brain

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Introduction: Type-1 diabetes mellitus (T1D) results from defective insulin secretion, with a consequent hyperglycemia, leading to a general derangement of cellular energy metabolism in target organs. In T1D a gradual development of complications in the central nervous system, defined as 'diabetic encephalopathy' occurs, that is characterized by neurophysiological and structural changes in brain and that, ultimately, results in impairment of cognitive function. Works on animal models, in particular streptozotocin (STZ) induced T1D in rats, provided evidences that diabetic encephalopathy is associated with mitochondrial dysfunction in different brain regions (1). Impaired mitochondrial oxidative phosphorylation, enhanced ROS level and altered mitochondrial structure have been observed.

Materials and Methods: Diabetes was induced in male Wistar rats by a single intraperitoneal injection (70 mg/kg) of streptozotocin and, after 3-weeks, brain mitochondria, from control and STZ-rats, were isolated. Mitochondrial proteins were analyzed using a high-throughput proteomics approach, employing zoomed two-dimensional gel electrophoresis (2DE), and subsequent mass spectroscopy (MS) identification. Western blot analysis by monoclonal antibody anti-NDUFS3 and Real-Time qPCR experiment was carried out. Spectrophotometrical catalytic activity of mitochondrial complex I was performed.

Results and Discussion: After 3-weeks STZ treatment, rats displayed the clinical hallmarks of diabetes. After image analysis, no changes were detected in the pI region 6–11 of the diabetic sample, while only one spot in the pI region 4–7 that showed a statistically significant down expression in the diabetic condition. This spot was digested and, subsequentially, identified by mass spectrometry analysis as NDUFS3, a 30 kDa protein of mitochondrial complex I. To confirm this result western blot analysis showed an analogue decreased NDUFS3 expression in diabetic rats and Real-Time qPCR experiment display that NDUFS3 mRNA amount was only slightly decremented. Moreover, spectrophotometrical catalytic activity of complex I showed a relevant decrease in the diabetic rat brain sample.

Our results (2) suggest the involvement of NDUFS3 in complex I deficiency in brain damage associated with the early stages of T1D.

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P05-23
Effect of N-acetyl cysteine on human testicular cancer cell membrane lipid profile

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Polyunsaturated fatty acids (PUFA) are major components of phospholipids, the principal structural unit of biological membranes. Unsaturated fatty acids have one or more double-bonds in a cis or trans configuration. Trans isomers are able to perturb both cell membrane arrangement and lipid enzymatic cascades. Endogenous trans fatty acid isomers are formed by the isomerization of fatty acids in cell membranes due to an endogenous free radical process. Reactive oxygen species (ROS) lead to the oxidative degradation of lipids in cell membranes, resulting in cell damage. Cell membranes, which are structurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and, consequent changes result in altered membrane fluidity, permeability, and cellular metabolic dysfunction. *N*-acetyl cysteine (NAC) is a potent anti-oxidant, can react directly with ROS and a source of cysteine for glutathione synthesis and increases levels of glutathione which is the major antioxidant of body. NAC has been shown to have protective effects towards DNA damage and carcinogenesis.

In this study, we identified the fatty acid profile of human testicular cancer cell membranes and examined the effect of NAC on the membrane fatty acid profile of these cells. The membrane fatty acid profile analysis in control and NAC treated samples were performed using gas chromatography (GC) with external reference standards. Membrane lipid were extracted and transesterification was performed to obtain the fatty acid residues as the corresponding methyl esters. Incubation of cells with 10 mM NAC for 24 hour, caused an increase in trans fatty acid concentration and saturated fatty acid percentage. Unlike to these findings, incubation with NAC decreased PUFA and monounsaturated fatty acids percentage in testicular cancer cell membranes.

P05r-24
Effects of n-3 PUFAs on structure and function of breast cancer lipid rafts

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Epidemiologic and experimental studies suggest that dietary fatty acids, in particular polyunsaturated fatty acids (PUFA), influence the development and progression of breast cancer. However, the mechanism by which n-3 PUFAs inhibit cancer cells growth, is not yet well understood. It was suggested that these FA might change the fluidity and structure of cell membrane, especially of lipid rafts.

Our data obtained by HPLC-GC analysis demonstrated that DHA and EPA are incorporated and metabolized in breast cancer cells. In particular it was also observed that EPA and DHA are incorporated in breast cancer lipid rafts with different specificity for the phospholipid moiety. Worth of note is the observation that only the treatment with DHA induces a reduction of Chol and SM content in lipid rafts, indicating a possible change in raft organization. Changes in the breast cancer lipid rafts structure were also analyzed with atomic force microscopy (AFM). The AFM analysis indicates a reduction of microdomains number, after DHA incorporation. Moreover PUFA treated rafts are dimensionally different from control rafts.

Taken together, our results indicate that n-3 PUFA 'feeding' might induce modifications of lipid rafts structure increasing the degree of FA unsaturation, and these changes might modify signal transduction and cell-cell interactions. Epidermal growth factor receptor (EGFR) is reported to concentrate in lipid rafts and is over expressed in breast cancer. Moreover, recent evidences show that estrogen receptor (ERα), that determines malignancy of (ER+) breast cancer, is membrane-associated. Our data demonstrate that n-3 PUFA incorporation determines lipid rafts biophysical changes and consequently is responsible of the modification in signal cascade of EGFR and ERα.

P05-25
Chimaeras of plant and microbial ion-translocating pyrophosphatases targeted to specific membrane systems efficiently recover H^+ and Na^+ homeostasis defects in yeast

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Inorganic pyrophosphate (PPi) is a ubiquitous energy-rich metabolite produced in many anabolic reactions, its efficient hydrolysis being required for them to occur in the biosynthetic direction. Ion-pumping membrane PPases, H^+(or Na^+)-PPases, couple PPi-hydrolysis, an essential biochemical reaction (EC 3.6.1.1), to the generation of transmembrane ion gradients, a versatile form of biological energy. They conform the simplest class of primary ion pumps known to date (homodimers, ca. 70 kDa subunits), allowing the use of PPi as a 'low-cost' energy currency alternative to ATP. Our group optimised the heterologous expression of a number of microbial and plant membrane PPases in *Saccharomyces cerevisiae* by manipulating their N-terminal domains with appropriate signal peptides, and further addition of the yEGFP coding sequence between the N-terminal and PPase sequences. These N-terminal domains changed the subcellular distribution of the resulting protein chimaeras, directing them to specific cell membranes. This experimental set-up has allowed us to carry out *in vivo* studies in yeast that tackle important issues such as the functional complementation of V-ATPase by plant H^+-PPases directed to the endomembrane system and eventually to the vacuole, as well as the enhanced sodium tolerance of a hypersensitive Ena1-4-deficient strain by an archaeal Na^+-PPase targeted to the cell plasma membrane. On the whole, our results demonstrate the ability of membrane PPase to generate by itself physiologically competent ion gradients *in vivo*, as well as its full capacity to functionally complement more complex ATP-dependent ion pumps. Moreover, they have raised new ways of engineering stress tolerance, and outlined a suitable experimental system to study alternative functions of their ATP-driven counterparts.

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P05-26
How mitochondrial potassium channels are regulated by polyunsaturated fatty acids

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Polyunsaturated fatty acids (PUFA) and their metabolites, have been implicated in the modulation of a variety of biochemical processes in the cell, and also in prevention of various diseases. They were able to interact with the membrane proteins including ion channels in normal and pathological conditions. The final goal of the project was to develop a description of the mechanism, that is responsible for activity modulation of neuroprotective mitochondrial potassium channels by selected PUFAs. The aim of the experiments was to determine whether the neuroprotective mechanism of the large-conductance calcium activated potassium (mitoBK$_{Ca}$) channel from the inner mitochondrial membrane can be explained by mutual interaction between the channels and polyunsaturated fatty acids.

The study was performed using patch-clamp technique and mitochondria isolated from rat astrocytes. We analyzed effect of arachidonic acid (AA), eicosatetraynoic acid (ETYA) a non-metabolizable analog of AA, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The open probability of the channel did not change significantly after application of 10 μM ETYA, but after adding 10 μM AA, which suggest that channel is affected by metabolites of AA rather than fatty acids itself. Application of 30 μM DHA, the same as 10 μM EPA increased open probability of the channel. Also number of the open channels in the patch increased in the presence of 30 μM EPA.

Summarising, our results indicate that neuroprotective PUFAs, like DHA and EPA activate mitoBK$_{Ca}$ channel, while proapoptotic and proinflamatory AA had no effect on mitoBK$_{Ca}$ channel.

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P05-27
The functional analysis of NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8) and its iron-sulfur clusters in human mitochondrial complex I

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NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8) is one of the nuclear-encoded subunits of human mitochondrial complex I. It contains two tetranuclear iron-sulfur clusters and is believed to play an essential role in electron transfer. Mutations on NDUFS8 have been associated with Leigh syndrome, bipolar disorder and encephalomyopathy. In this study, we first applied RNA interference technique to knock down the expression of NDUFS8 in T-REx293 cells and investigate its effects on complex I functions. Our results demonstrated that reducing expression of NDUFS8 in cells would retard the cellular growth rate in galactose-only media, reduce complex I enzymatic activity and oxygen consumption efficiency, and increase the production of reactive oxygen species (ROS). In addition, these NDUFS8-suppressed cells showed a decrease in ATP generation and compromised the mitochondrial membrane potential. Moreover, knockdown of NDUFS8 affected not only the intactness of complex I but also the assembly of complex III. These results indicated that NDUFS8 subunit plays a crucial role in maintaining complex I structure and functions. To investigate the importance of iron-sulfur cluster motifs on mitochondrial import of NDUFS8, a series of mutations on the conserved cysteine residues in this protein were generated and their effects on protein localization were explored. In contrast to their corresponding cysteine mutations in *Rhodobacter capsulatusand Neurospora crassa* homologues, replacement of the conserved cysteine residues in the iron-sulfur clusters did not affect the NDUFS8 targeting to mitochondria and the assembly of complex I. Unexpectedly, we also found that most of the N-terminal deletion constructs of NDUFS8 protein were located in a specific region of nuclei.

P05-28
Sterols modulate vacuolar proton transport and autophagy in yeast

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Inhibitors of the sterol-Δ^8, Δ^7-isomerase (*ERG2* gene in yeast) are widely used as fungicides and comprise a family known as amine fungicides. Recently, it has been proposed that V-ATPase inhibition is behind the fungicidal effect of the closely related azole fungicides. However, the cellular mode of action of amine fungicides is still debatable. Here we show that abnormal Δ^8-unsaturated sterols inhibit proton transport by negatively affecting vacuolar H^+-ATPase functionality in yeast. Accordingly, cells defective in *ERG2* show V-ATPase-like phenotypes such as sensitivity to alkaline pH or zinc. Plants typically show a double set of electrogenic proton pumps at their vacuoles and are resistant to these fungicides. Expression of an alternative proton pump, a chimaera of the plant vacuolar H^+-pyrophosphatase *AVP1*, efficiently complements V-ATPase-like defects in *erg2*$^-$ mutants. Tridemorph, an *Erg2p*-specific fungicide, induces V-ATPase defects even at low concentrations. However, V-ATPase inhibition is not the cause of cell death since *erg2*$^-$ mutants can grow under standard conditions, while fungicide-treated wild-type cells die by apoptosis. A consequence of V-ATPase inhibition are defects in the process of autophagy akin to those found in V-ATPase mutants. Being this a paramount process to maintain chronological lifespan, we have done a series of experiments to assess the importance of proton transport in *erg2*$^-$ mutants.

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P05-29
Alternative electron carriers in photosynthesis

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Most organisms performing oxygenic photosynthesis contain either cytochrome c_6 (Cc_6) or plastocyanin (Pc), or both, to transfer electrons from cytochrome b_6-f to photosystem I (PSI). However, it has been described that the cyanobacterium *Synechocystis* is able to grow photoautotrophically in the absence of both Cc_6 and Pc, opening the door to the existence of alternative pathways in the photosynthetic electron flow.

The soluble electron carriers Cc_6-like, its counterpart in plants Cc_{6A}, and cytochrome c_M, are proteins phylogenetically related with Cc_6. The physiological role of these three cytochromes remains however unknown, and might be associated with alternative electron transport reactions, detoxification, stress tolerance, or even nitrogen fixation. These proteins could be potentially used to divert the energy obtained in photosynthesis or respiration to other metabolic productive routes.

Several *Synechocystis* mutants that grow photoautotrophically in the absence of Cc_6 and Pc have been characterised. In addition, we have cloned and overexpressed the *cytA* gene from the cyanobacterium *Nostoc* sp. PCC 7119, which encodes for a Cc_6-like that could act as an alternative carrier. The sequence analysis showed that the protein is located inside the thylakoidal lumen and possesses a surface area homologous to the Cc_6 hydrophobic area interacting with PSI and cytochrome *f*. Cc_6-like is able to react with PSI, although less efficiently that Cc_6 and Pc. However, the surface electrostatic potential distribution and redox potential suggest that Cc_6-like would not be able to accept electrons from cytochrome *f*. Thus, the physico-chemical and functional analysis carried out has shown that the Cc_6-like cannot replace Cc_6 and Pc in the electron transfer from cytochrome *f* to PSI.

P05-30
Dab2 and low density lipoprotein receptors (LDLR) in rat small intestine

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Dab2 is an intracellular adaptor protein that directs the endocytosis of members of LDLR family. This family includes megalin, ApoER2 and VLDLR, which are located at the apical membrane of the intestinal epithelium (1,2). We have proposed that Dab2

mediates the endocytosis of milk macromolecules (3), but nothing is known on the receptors that interact with Dab2 in the small intestine. Herein we investigate whether the receptors above mentioned act with Dab2 to mediate intestinal endocytosis during the lactation period.

All procedures were accord with current national/local ethical guidelines. Enterocytes and crypts were isolated from jejunum and ileum of rats of different ages. Real-time PCR and immunolabeling techniques have been used.

Of the three receptors under study, only the expression pattern of megalin is similar to that of Dab2. For both genes, mRNA and protein abundance is higher in ileum that in jejunum, down-regulated by age and up-regulated by components of the milk. Furthermore, megalin, cubilin and amnionless are co-localized with Dab2 at the apical endocytic apparatus of the enterocytes. Dab2 also co-localizes with VLDLR and with ApoER2 at the apical endocytic apparatus. However, expression of ApoER2 is regulated neither by age nor by milk diet. And VLDLR expression in the enterocytes and crypts is differently affected by age and, if any, it is down-regulated by milk diet.

We conclude that in the intestine Dab2 may act as an endocytic adaptor for (i) the megalin/cubilin/amnionless complex receptor during lactation and (ii) ApoER2 and VLDLR mediated processes.

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P05-31
Tethering of lipid membranes reorganizes the distribution of lipid domains

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Characterization of phase coexistence in biologically relevant lipid mixtures is often carried out through confocal microscopy of giant unilamellar lipid vesicles (GUVs), loaded with fluorescent membrane probes. This last analysis is generally limited to the vesicle hemisphere further away from the coverslip, in order to avoid artifacts induced by the interaction with the solid surface, and immobilization of vesicles is in many cases required in order to carry out intensity, lifetime or single molecule based microscopy. This is generally achieved through the use of membrane tethers adhering to a coverslip surface. Here, we aimed to determine whether GUV immobilization through membrane tethers, induces changes in lipid domain distribution within liposomes displaying coexistance of lipid lamellar phases. Confocal imaging and a Förster resonance energy transfer (FRET) methodology showed that biotinylated phospholipids present significantly different membrane phase partition behavior upon protein binding, depending on the presence or absence of a linker between the lipid headgroup and the biotinyl moiety. Membrane phases enriched in a membrane tether displayed in some cases a dramatically increased affinity for the immobilization surface, effectively driving sorting of lipid domains to the adherent membrane area, and in some cases complete sequestering of a lipid phase to the interaction surface was observed. On the light of these results, we conclude that tethering of lipid membranes to protein surfaces, has the potential to drastically reorganize the distribution of lipid domains, and this reorganization is solely dictated by the partition properties of the protein-tether complex.

P05-32
Oxidized heme – a novel inhibitor of calcium-regulated BK channel in rat brain mitochondria

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Heme is a prosthetic group that consists of an iron atom bound in the center of a porphyrin ring. It is an essential element of hemeproteins in all living organisms. Although heme is ubiquitous, its circulation is strictly controlled. Therefore it is believed that it may play as yet unknown regulatory functions.

The mitochondrial calcium-dependent BK channel (mitoBK_{Ca}) is one of the five known channels that contribute to potassium permeability of mitochondrial inner membrane. It is activated by calcium and voltage and inhibited by scorpion venom toxins such as charybdotoxin and iberiotoxin.

In the current study, we have checked the impact of the oxidized heme (hemin) on mitochondrial membrane potential and respiration rate of rat brain mitochondria. We have shown that hemin prevents the collapse of membrane potential that is normally caused by calcium-dependent BK channel openers (NS1619). A similar, though modest effect was observed in studies of oxygen consumption rate. We also report inhibitory effects of hemin on the reactive oxygen species-downregulating protperties of NS1619.

Additionally, we have studied the single channel activity of mitoBK_{Ca} by patch-clamp of mitoplasts isolated from a rat astrocyte cell line. The results that we have obtained confirm the phenomonon of reversible inhibition of mitoBK_{Ca} channel by hemin.

Our findings support the hypothesis that oxidized heme can inhibit the mitochonbdrial calcium-dependent BK channel.

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P05r-33
Phospholipid Scramblase 1: C-terminal peptides insertion studies and influence of the lipid environment

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Human Phospholipid Scramblase 1 (hPLSCR1) is a plasma membrane intrinsic protein when multipalmitoylated (1), that promotes calcium-dependent phospholipid translocation. Neither the calcium-induced conformational change nor the mechanism of phospholipid scrambling are known in detail. There are even many doubts about its intrinsic anchorage to the membrane; (2) suggests that the unique alpha-transmembrane domain, localized at the C-terminal region, remains buried in the protein core.

We are working with hPLSCR1 C-terminal region peptides to probe their adsorption/ insertion into model membranes. We also want to check the modulation of hPLSCR1 activity by the lipid environment architecture, in the presence of long-chain ceramides or lysophospholipids.

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P05-34
Organization of the secretory pathway of polarized fungal cells: change of membrane identity by maturation from the early Golgi to the Spitzenkörper

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Over the last years, cell biology studies in the filamentous fungus *Aspergillus nidulans* have established a firm correlation between the strong polarization of the ER exit sites and the Golgi equivalents in the tip cells with high apical extension rates (1), making the system ideally suited for genetic and cell biological studies of the secretory machinery.

By multidimensional *in vivo* fluorescence microscopy we showed that the Golgi Equivalents (GEs) mature, such that Sed5/Rer1 (early) cisternae are continuously being transformed into PI4P/Tlg2 (late) ones. Near the apex, late GEs increase their size and undergo fission, giving rise to new cisternae, which may account for the fact that the late Golgi is more polarized than the early Golgi. Late GEs mature to Rab11-positive post-Golgi carriers that undergo movement towards the apex, using both microtubules and actin cables. This movement targets post-Golgi membranes to the Spitzenkörper (SPK), a specialized, secretion-related structure of filamentous fungi located underneath the apical plasma membrane. Upon drug-induced depolymerization of the cytoskeleton, Rab11 membranes accumulate in the proximity of the Golgi. Motile post-Golgi membranes do not overlap with RabARab5 endosomal membranes. The SPK localization of Rab11 is dependent on the exit from the Golgi, as shown with the Sec7-inhibitor Brefeldin A and by using a Sec7 ts mutation (hypB5). Rab11 is essential, while Rab8 is not, although rab8Δ affects hyphal shape and growth rate. rab8Δ and rabC(rab6)Δ^2 show a severely debilitating synthetic effect. We propose a model where Rab11 regulates the accumulation of post Golgi carriers in the SPK, whereas Rab8 and Rab6 regulate the precise localization and efficiency of fusion of these carriers with the plasma membrane.

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P05-35
Alkylphospholipids and cholesterol metabolism in glioblastoma cell line U87-MG

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Alkylphospholipid (APL) analogues are a family of synthetic lipid agents with antitumor and antiparasitic activities.

In previous works, we established that APLs alter intracellular cholesterol metabolism leading to an increased uptake and biosynthesis of this sterol in the human hepatoma cell line HepG2. Moreover, we used radioactive labelled lipids to report that cholesterol trafficking from plasma membrane to endoplasmic reticulum is disrupted by APLs.

We decided to extend these studies to glioblastoma (U87-MG) cell line. In this work we show that APLs exhibit more antiproliferative activity in gliobastoma cell line than in HepG2 cells. We detected cell cycle arrest by flow cytometry in APLs treated cells. We show that APLs inhibit cholesterol trafficking and, using the fluorescent cholesterol marker filipin, we visualized intracellular accumulation of this sterol. These results are interesting in order to clarify how APLs can affect the internal dynamic of lipid uptake, trafficking, accumulation and metabolism. This knowledge is interesting regarding the connection between antitumor activity and lipid deregulation and can be useful in cancer therapy for future.

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P05r-36
TcP5CDH modulates mitochondrial respiratory chain in the protozoan specie *Trypanosoma cruzi*

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T. cruzi, the protozoan causing the Chagas disease, uses L-proline as a main energy source. delta-1-pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) is the second enzyme of the proline-breakdown pathway, catalysing the conversion of P5C into glutamate. In *T. cruzi* we have seen that *Tc*P5CDH is bound to mitochondrial membranes. The recombinant *Tc*P5CDH was obtained and purified to homogeneity, and kinetic data were determined for its activity in both, the recombinant form and extracts of mitochondrial-enriched vesicles. Upon a bioinformatic analysis, a single putative trans-membrane domain (F198-K221), which presumably spans mitochondrial membranes, was also predicted. SAXS measurements revealed that soluble *Tc*P5CDH is multimeric, being composed by ten monomeric sub-units with a whole molecular weight of 629.7 kDa. SAXS data analysis suggested a radius of gyration of 65.21 ± 0.53 Å with a maximum diameter of 200 Å. The participation of *Tc*P5CDH in respiratory chain processes was also approached. Then, permeabilized stable mutant epimastigotes over-expressing an ectopic copy of *Tcp5cdh*, upon succinate stimulation presented higher oxygen uptake (up to 40%) when compared to wild-type cells. Similar results were obtained by using L-proline or DL-P5C as respiratory substrates. Unexpectedly, in these conditions, the addition of ADP was not able to stimulate oxygen consumption, suggesting that both substrates could affect mitochondrial respiration. The assumption that P5CDH should interact physically with cytochrome *c* oxidase complex, as seen in proteomic analysis and as reported for *T. brucei*, is currently being studied. In addition, the effect of a reported inhibitor for ALDHs (DSF) was tested in distinct parasite life stages, showing a strong trypanocidal effect in proliferative (IC_{50} = 650 nM) and infective forms. Conversely, when cells over-expressing *Tcp5cdh* were grown in the presence

of DSF, those exhibited a higuer resistance rather than in wild-type cells. Our data postulate *Tc*P5CDH as an essential enzyme involved in the bioenergetics of *T. cruzi*.

P05-37
Setting up a benchmark for the characterization of TRP channels

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TRPV1 and TRPV2 are members of the superfamily of the Transient Receptor Potential (TRP) ion channels. They are assembled into homotetramers and allow cations across the membrane in response to stimuli such as heat (TRPV1 and TRPV2) and capsaicin (TRPV1). TRPV1 and TRPV2 share ~50% sequence identity, however the pharmacology difference profiles for TRPV1 and TRPV2 are not so well understood, in fact TRPV2 is an orphan receptor, since no specific endogenous ligand has been identified yet. We propose a multidisciplinary approach to study these channels. To better understand TRPV1 and TRPV2 roles and to go further into their structure, large-scale protein expression of the active forms is required. Here we present our advances in the protein expression and purification of active TRPV1 and TRPV2. In addition, to infer significant hints about the role of TRPV2 and to go further into its function, sequence analysis of orthologs of TRPV2 has been carried out to define common and differential functional/architectural regions. Preliminary biophysical characterization such as thermal stability, and secondary structure composition analysis has been carried out on TRPV2 to identify key structural points in the TRPV2 topology. In addition, chemoinformatics analysis has been performed to identify relevant pharmacological seeds to develop a drug design approach.

P05-38
The histidine connection with the Krebs cycle in *Trypanosoma cruzi*: uptake and firsts steps of the histidine – glutamate pathway

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Different roles in the biology of trypanosomatids were reported for several amino acids beyond their well- known participation in protein synthesis and energy metabolism. This is the case of histidine, which was described as an antioxidant and anti-inflammatory agent. In most of eukaryotic cells, histidine is degraded to glutamate in a non-oxidative pathway involving four enzymatic steps. No genes for histidine degradation were identified in trypanosomatids with the only exception of *T. cruzi*. However, little is known about this pathway in these cells. In the present work we present a first approach the the histidine to glutamate pathway in *T. cruzi*. We firstly characterized the uptake of histidine as occurring through a single saturable transport system (K_M = 0.25 mM, V_{max} = 0.44 nmoles per minute $\times 2 \times 10^{-7}$ cells). The system was shown to be slightly dependant on pH and ATP levels. Once inside the cells, histidine is converted into urocanate and ammonia by the enzyme histidine ammonia-lyase (HAL, EC 4.3.1.3). We cloned the putative gene for this enzyme (TcHAL) and, after expression and purification, it was biochemically characterized (K_M = 1.03 mM, V_{max} = 2.54 μmoles/min/mg). We observed that TcHAL is dependent on divalent cations and that is expressed as a cytoplasmic protein in all the stages of the life cycle of *T. cruzi* tested. The second enzymatic step is the conversion of urocanate in 4-imidazolone-5-propionate, catalyzed by the enzyme urocanase (EC 4.2.1.49). We also cloned the putative gene for this enzyme (TcUH) and the recombinant protein was expressed and purified. Preliminary, the kinetic parameters were obtained from crude extracts of epimastigotes (K_M = 12 μM, V_{max} = 10.4 nmoles/min/mg) which are being validated by measuring the activity with the recombinant enzyme. Presently, we are evaluating the role of this pathway in the resistance against oxidative stress. The ability of this pathway to connect main metabolic routes in *T. cruzi* prompts these enzymes as new therapeutic target against this organism.

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P05r-39
Cytochrome c_1 – cytochrome *c*: an atypical transient complex

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The membrane-embedded cytochrome bc_1 complex (bc_1) donates electrons to soluble cytochrome *c* (C*c*) in mitochondrial respiration, with cytochrome c_1 (Cc_1) acting as the direct subunit interacting with C*c*. The electron transfer (ET) complexes do usually require a relatively low affinity between partners, so as to guarantee their high turnover. Co-crystallization of the two partners thus becomes rather uncommon. One of the few exceptions is the yeast bc_1-C*c* complex, whose crystal structure was actually solved (1). Even though many features of such a complex were addressed in the crystal structure, its physiological relevance and functioning should be supported by binding assays in solution. Here, we use Cc_1 devoid of its C-terminal transmembrane α-helix to analyze the Cc_1-C*c* contacts by Nuclear Magnetic Resonance (NMR) and Isothermal Titration Calorimetry (ITC).

Strikingly, NMR titrations reveal that the C*c* amide signals are in fast/intermediate exchange upon binding to Cc_1, so yielding a well-defined surface on C*c* – the most disturbed residues are surrounding the heme crevice. Nevertheless, the chemical shifts of C*c* resonances caused by Cc_1 binding are significantly larger than those observed in another respiratory complex, namely the C*c*-cytochrome *c* oxidase complex (2), thereby suggesting that the Cc_1-C*c* adduct is longer-lived in solution. This is in agreement with the value we have determined by ITC for the dissociation constant of the Cc_1-C*c* complex (3 μM). Indeed, our NMR restraint-driven docking computations show a single, well-defined Cc_1-C*c* conformation, which is compatible with an efficient ET reaction. Altogether, our findings provide an in-depth understanding of the Cc_1-C*c* interaction, an ET model system that behaves as an atypical transient complex in solution.

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P05-40
Edelfosine and miltefosine fluidize lipid rafts: implications for Fas-mediated apoptosis triggering

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Edelfosine (1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-phosphocholine) and miltefosine (hexadecylphophocholine) are representative molecules of synthetic alkylphospholipids, which possess a potent cytotoxic activity against several tumors. These ether lipid analogues are selectively accumulated in lipid rafts of tumor cells, inducing Fas (CD95/APO-1) clustering and the recruitment of downstream apoptotic proteins into these regions, thus initiating apoptosis. The exact mechanism by which ether lipids trigger rafts and apoptotic molecules coalescence is still not fully understood. Recent evidences support that the modulation of lipid rafts biophysical properties by these drugs might be underneath their mode of action. We have studied the effects of clinically relevant amounts of edelfosine and miltefosine in the properties of mammalian raft model membranes composed of an unsaturated glycerophospholipid, sphingomyelin and cholesterol. The influence of these drugs on membrane order and fluidity, lateral organization, lipid rafts amount and sizes were studied by employing a multiprobe steady-state and time-resolved fluorescence approach, Förster resonance energy transfer (FRET) and microscopy. Our results show that the alkyl ether lipid localization into rafts does not result solely from their interactions with cholesterol. Moreover, both edelfosine and miltefosine increase the fluidity of lipid rafts, which might facilitate Fas translocation to those regions. These results are discussed within the picture of alkyl ether lipids-mediated cell death.

P05-41
Altered surface electrical charge in erythrocytes by vipoxin from *Vipera ammodytes meridionalis* and lectins

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Vipoxin is a heterodimeric neurotoxic complex composed of a strongly toxic phospholipase A_2 component (PLA_2) and an acidic non-toxic and catalytically inactive component (VAC), isolated from *Vipera ammodytes meridionalis* venom. According to our purpose, vipoxin provided a valuable tool for exploring the membrane electrostatic and electrokinetic phenomena. Here, we present for the first time altered electrokinetic properties of human erythrocytes treated with both vipoxin complex and its separated individual subunits – PLA_2 and VAC. Electrophoretic mobility (EPM) was measured by microelectrophoresis with an OPTON Cytopherometer (Austria). We compared erythrocytes after treatments with: 1. pure PLA_2 and VAC; 2. vipoxin; and 3. reconstituted complex (pre-incubated mix of pure subunits). Compared to the untreated erythrocytes, our results showed higher net negative EPM and zeta (ε) potential due to the increased negative electrical charge on the outer surface of the membranes at different concentrations of proteins used. The influence of lectins (wheat germ agglutinin, phytohemagglutinin PHA-S, peanut agglutinin) on the ε potential of cells was also studied. There was an enhancement in ε potential of erythrocytes upon treatment with lectins. The significant differences in erythrocytes' surface charge change indicated the promising role of ε potential as a marker of stability in studying the cellular interactions of vipoxin and lectins with biomembranes.

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P05-42
The yeast Ptc7p mitochondrial phosphatase, the crossroad of coenzyme Q biosynthesis, mitophagy activation and chronological life span extension

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Coenzyme Q (CoQ or Q) is an essential isoprenylated benzoquinone component of mitochondria, which functions mainly as an electron carrier from complex I, or II to complex III at the inner membrane, and as an antioxidant particularly on lipoproteins and plasma membrane. CoQ biosynthesis is a highly regulated process driven by a multi-protein complex that catalyzes the modifications of the benzene ring. Coq7p/Cat5p (Coq7p) catalyzes one of the latest steps required for the final conversion of the late intermediate demethoxy-Q_6 (DMQ_6) to Q_6, which also represents a key regulatory step in this pathway. Coq7p dephosphorylation was produced by the mitochondrial Ptc7 protein Ser/Thr phosphatase that activates aerobic yeast metabolism by regulating coenzyme Q (CoQ) biosynthesis. Yeast lacking *PTC7* (*YHR076w*) gene exhibited decreased of both mitochondrial function and oxidative stress defenses, leading to increased protein carbonylation damage. CoQ content was decreased in *PTC7* deleted strain, suggesting that during respiratory metabolism Ptc7p activates the CoQ_6 biosynthesis. Ptc7p dephosphorylates Coq7p in both *in vivo* and *in vitro* assays. *PTC7* null mutant exhibited increased Coq7p phosphorylation when CoQ biosynthesis was induced. Chronological life span (CLS) is defined as a survival mechanism that depends on metabolic and stress adaptations to environment. *PTC7* strain showed a decreased CLS that was not rescued by exogenous CoQ_6. Rescue of CLS required Ptc7p that also activated mitophagy but not macroautophagy. These results led us to propose that Ptc7p links homeostasis of CoQ by regulating its biosynthesis through the phosphorylation stage of Coq7p and mitochondrial recycling as an adaptation mechanism to both stress and nutritional environment changes to promote CLS.

P05-43
Steady-state fluorescence anisotropy analysis of canonical lipidic mixtures involved in membrane heterogeneity

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Cholesterol constitutes from 30 to 50% of the lipid mass in plasmatic cell membranes, and modulates physicochemical properties

of lipid bilayers, such as the ordering of acyl chain region, the increasing the thickness of the hydrophobic region, the decrease the area/volume ratio, as well as polarity, fluidity and permeability.

Although there are some 1-palmitoyl-2-oleoyl-3-*sn*-glycerophosphocholine (POPC)/cholesterol (Chol) and egg-sphingomyelin (egg-SM)/cholesterol thermal phase diagrams available in literature, they are not unequivocally and definitely established. Clarifying how addition of cholesterol, at certain proportions, affects the lipid bilayers fluidity, is of utmost importance to evaluate physicochemical properties that modulate biological membranes, varying between normal and pathological states, is the main goal of this work.

The effect of addition of cholesterol, at certain concentrations, in lipid bilayers composed of binary mixtures consisting of POPC + Chol and egg-SM + Chol; and ternary mixtures consisting of: POPC + egg-SM + Chol, was determined by steady-state fluorescence anisotropy, using DPH as fluorescent probe. The results are analyzed applying quantitative approaches to evaluate the anisotropy dependency upon temperature (linearization of exponential and double derivative of sigmoidal variation curves, applied to unsaturated and saturated phospholipids, respectively) and are useful to improve and clarify the referred phase diagrams.

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P05r-44
Cytochrome c_1: an old protein with novel functions

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Cytochrome c_1 (Cc_1) is a well-known membrane-bound subunit of the mitochondrial cytochrome bc_1 complex that transfers electrons to cytochrome *c* (C*c*) in cellular respiration. Meriting particular interest is the dual targeting of plant Cc_1, which is located not only in mitochondria but also in chloroplasts, albeit its plastidial function still needs to be explored (1). Recently, new roles of Cc_1 in cell fate have arisen. In particular, it has been reported that the soluble domain of Cc_1– devoid of its C-terminal transmembrane α-helix – triggers cell death events, thus serving as a pro-apoptotic marker (2,3). To shed light on such novel functions of Cc_1 at the molecular level, we have designed and expressed a soluble, truncated form of plant Cc_1. The proper folding of the recombinant protein, as well as its heme coordination and redox potential have been validated by combining Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR); the monomeric state of Cc_1 has been corroborated by analytical ultracentrifugation; and a homology model has been built with the MODELLER software package using the crystallographic structures of bovine, chicken and yeast Cc_1 as templates. Work is now in progress to further explore the novel functions of plant Cc_1, namely its peroxidase activity and its ability to trigger the caspase cascade activation. Financial support from the Ministry of Economy and Competitiveness (BFU2009-07190, and FPU Fellowship AP2009-4805) and Andalusian Goverment (BIO-198).

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P05-45
Pyrene and pyrenyl connections: diffusion-controlled reactivity, equivalent polarity and amphiphilic cohesiveness in lipid bilayers

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Pyrene and pyrenyl probes offer diverse advantages, such as high quantum yields, long lifetimes, and solvatochromic effects, most appropriate for fluorescence studies in biological systems. They have been therefore extensively used to probe varied phenomena in lipid bilayer biophysics. We used pyrene to monitor the equivalent polarity of binary mixtures of phosphatidylcholines (DPPC, POPC, DOPC and egg-sphingomyelin – egg-SM) and cholesterol (Chol), probed by the pyrene Ham Effect. For POPC and DOPC the polarity values tend to decrease (either increasing Chol concentration or temperature). For DPPC and egg-SM, we verified the same tendency for low Chol contents, but for high Chol proportions and higher temperatures, we observe an increase in the equivalent polarity, mostly for egg-SM.

We used β-py-C_{10}-HPC and β-py-C_6-HPC pyrenyl labeled phospholipids in POPC vesicles to examine the excimer formation process by combined steady-state and lifetime fluorescence. Our findings are in very good agreement with the theoretical predictions of a kinetic formalism for fluorescence quenching processes occurring in two-dimensional media. However, a significant downward divergence occurs above 4 mol % of probe content, which might indicate deviations to the presumed lateral diffusion consistency of the probes. We extended the analysis to POPC/Chol mixtures, putatively in the liquid-ordered (35, 40 and 45 mol % cholesterol) and in liquid-disordered (5 mol %) phases, and compare the outcomes with the results from pure POPC bilayers, by using the intramolecular excimer formation exhibited by pyrenyl phopholipids labeled in both fatty acid chains, noticing diferential variations in the cohesiveness of lipid bilayers.

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P05-46
Polyglutamine aggregates can cause mitochondrial hyperplarization in yeast *Saccharomyces cerevisiae*

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Earlier we have shown that expression of an expanded N-terminal polyglutamine domain of human huntingtin (polyQ) in yeast *S. cerevisiae* interferes with the cell cycle. Here we showed that an abrupt termination of the expression is also damaging for cells. We found that the change of the carbon source contributes to the toxicity of the expression termination (polyQ is expressed from the Gal-promoter, and to stop the expression the cells are transferred from galactose to glucose). It appeared that polyQ-producing cells (but not the wild type) after transfer from galac-

tose to glucose hyperpolarize their mitochondria. Addition of the uncouplers reduces both the hyperpolarization and the toxicity caused by the transfer. We observed that a mutation in ubiquitine-proteosomal system (cdc53-1) has similar effect: mitochondrial hyperpolarization after galactose-to-glucose transfer. It is known that the cells rely either mostly on respiration or mostly on glycolysis for energy production. We speculate that polyQ aggregates overload ubiquitine-proteosomal system, which leads to inability of the cells to suppress respiration while activating glycolysis during transfer to glucose.

P05-47
VPS4, an ATPase involved in the biogenesis of multivesicular endosomes, is necessary for the deformation of the acrosomal membrane during acrosomal exocytosis in human spermatozoa

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The acrosome reaction of human spermatozoa is a complex, calcium-dependent regulated exocytosis. Fusion at multiple sites between the outer acrosomal membrane and the cell membrane causes the vesiculation of theses membranes and the release of the acrosomal contents. We have reported previously that by preventing the release of calcium from the acrosome, the exocytic process can be arrested at a stage where SNARE proteins are assembled in loose trans complexes. Transmission electron micrographs of sperm at this stage showed that the acrosomes were profusely swollen, with deep invaginations of the outer acrosomal membrane. The protruding edges of these invaginations were tightly apposed to the plasma membrane. We have proposed that these membrane deformations are part of the mechanism of vesiculation. Invagination of the acrosomal membrane is topologically equivalent to the formation of internal vesicles in endosomes, a process that depends on the assembly of membrane-bending ESCRT complexes on the endosomal surface. We are exploring the possibility that the same mechanism is involved in acrosomal exocytosis. A dominant-negative mutant of VPS4, the ATPase responsible for the disassembly of the membrane attached ESCRT proteins inhibited acrosomal exocytosis of permeabilized human spermatozoa. Moreover, an anti-VPS4 antibody was also inhibitory. TEM images show abnormal bending of the acrosomal membrane when sperm were stimulated in the presence of the dominant negative VPS4. These observations suggest that the deformation of the acrosomal membrane necessary for acrosomal exocytosis are shaped by an ESCRT-dependent mechanism.

P05-48
Functional relationship between Tau and Ca^{2+} transporters in adult and aging brain

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The Ca^{2+}-ATPases are membrane transporters that use the energy of ATP hydrolysis to pump cytosolic Ca^{2+} out of the cell (plasma membrane Ca^{2+}-ATPase, PMCA) or into internal stores (endoplasmic reticulum Ca^{2+}-ATPase, SERCA and secretory pathway Ca^{2+}-ATPase, SPCA). These pumps are the main high-affinity Ca^{2+} system involved in the maintenance of intracellular free Ca^{2+} at the properly low level in eukaryotic cells. Besides, they are implicated in localized Ca^{2+} signaling. Ca^{2+} dysregulation seems to be involved in brain aging and neurodegenerative diseases. One of these diseases that is closely related to aging is Alzheimer's disease. It is characterized by the accumulation of β-amyloid senile plaques and neurofibrillary tangles of tau, a protein that play key roles in axonal transport. In this work we have used kinetic and immunological assays to look for a functional relationship between tau and Ca^{2+}-ATPases in adult and aged brain. Western blots and Immunohistochemical analysis reveal higher expression of phosphorylated tau and distribution changes with aging, increasing the signal in the hippocampal CA1 region of 15-month old mice with respect to 3-old mice. Kinetic assays indicate that tau inhibits the Ca^{2+}-ATPase activity, decreasing this inhibition with aging. These results point out a link of Ca^{2+}-transporters with aging and neurodegeneration mediated by tau protein.

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P05-49
Interaction of amphipatic α-helix AH2 of protein NS4B from Hepatitis C Virus with model biomembranes

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Hepatitis C virus (HCV) has a great impact on public health, affecting more than 170 million people worldwide since it is the cause of liver-related diseases. HCV entry into the host cell is achieved by the fusion of viral and cellular membranes, replicates its genome in a membrane associated replication complex, and morphogenesis has been suggested to take place in the endoplasmic reticulum (ER) or modified ER membranes. The variability of the HCV proteins gives the virus the ability to escape the host immune surveillance system and notably hampers the development of an efficient vaccine. HCV has a single-stranded genome which encode a polyprotein, cleaved by a combination of cellular and viral proteases to produce the mature structural proteins (core, E1, E2, and p7) and non-structural ones (NS2, NS3, NS4A, NS4B, NS5A and NS5B), the latter associated with the membrane originated from the ER in the emerging virus. NS4B protein from Hepatitis C Virus (HCV) is a highly hydrophobic protein inducing a rearrangement of endoplasmic reticulum membranes responsible of the HCV replication process. Different helical elements have been found in the N- and C- terminal domains of the protein, which seem to be responsible for many key aspects of the viral replication process. In this work we have carried out a study of the binding to and interaction with model biomembranes of peptide $NS4B_{AH2}$, segment corresponding to the N-terminal of the protein, previously identified as a membrane interaction domain. We show that $NS4B_{AH2}$ partitions into phospholipid membranes and its membrane-activity is modulated by lipid composition. These data would suggest that the $NS4B_{AH2}$ region of this protein might be involved in the membrane alteration which must occur in the HCV replication and/or assembly process.

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P05-50
Lipid membranes as possible targets for apoptotic bile acids

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Hydrophobic bile acids (BAs) such as deoxycholic acid (DCA) induce cell death via multiple pathways, including the extrinsic pathway of apoptosis, dependent on pro-apoptotic receptor activation in the plasma membrane, and the intrinsic pathway of apoptosis, involving the release of pro-apoptotic factors from mitochondria. In turn, it has been shown that hydrophilic BAs, particularly ursodeoxycholic (UDCA) and tauroursodeoxycholic (TUDCA) acids, significantly inhibit most of these events, thereby preventing apoptosis. Still, the mechanisms by which BAs trigger such opposite signaling effects remain unclear. We hypothesize that cellular membranes may constitute a determinant primary target for the modulatory effects of BAs during apoptosis.

In this work, two nitro-benzoxa-diazol (NBD) fluorescent derivatives of DCA and UDCA, namely DCA-NBD and UDCA-NBD were studied. Both DCA-NBD and UDCA-NBD represented good mimics of unlabeled BAs, since both interacted with micellar and pre-micellar aggregates of the unlabeled molecules. In addition, both probes partitioned effectively and in similar fashion to model membrane systems (POPC liposomes) and were sensitive to the presence of cholesterol in the membrane. Interestingly, unlabeled hydrophilic BAs UDCA and TUDCA interacted with DCA-NBD at pre-micellar concentrations. These interactions did not change DCA-NBD partition properties. However, they could be associated with the observed anti-apoptotic effect of more hydrophilic BAs.

In conclusion, unlabeled BAs DCA, UDCA and TUDCA were shown to induce changes in the fluorescence properties of superficially-located membrane probes, which indicate a superficial but strong interaction with membranes.

P05-51
Interaction of a peptide derived from dengue virus protein C with membrane model systems

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There are three genera in the *Flaviviridae* family: *Flavivirus, Hepacivirus and Pestivirus*. Dengue virus (DENV) is sorted into the *Flavivirus* genus and it is the leading cause of arboviral diseases in the tropical and subtropical regions, affecting 50–100 million people every year. DENV is a positive-sense, single-stranded RNA virus with approximately 10.7 kb. It contains untranslated regions both at the 5' and 3' ends, flanking a single open reading frame (ORF) encoding a polyprotein of over 3000 amino acids, which is subsequently cleaved by cellular and viral proteases into three structural proteins, C, prM and E, and seven nonstructural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

The C proteins of *Flaviviridae* are dimeric, basic, have an overall helical fold and are responsible for genome packaging. These proteins are essential for viral assembly in order to ensure specific encapsidation of the viral genome. Protein C seems to associate with intracellular membranes through a conserved hydrophobic domain. Recently, it has been found that protein C accumulates around endoplasmic reticulum (ER) derived lipid droplets. Similarly to other enveloped viruses, DENV replicates its genome in a membrane-associated replication complex, and morphogenesis and virion budding has been suggested to take place in the ER or modified ER membranes. These modified membranes could provide a platform for capsid formation during viral assembly. Although *Flaviviridae* C proteins are shorter than the *Hepacivirus* core proteins, their roles should be similar as well as their capacity to bind to phospholipid membranes. From the analysis of structural parameters, dimers of protein C (readily formed in solution) contain a highly hydrophobic region comprising the a2 helices of each of the monomers. This region also forms a concave groove, providing an energetic argument for membrane association. In a previous work, we have found out a region that induced virtually 100% membrane rupture of several membrane model systems, which was coincident with theoretically predicted highly hydrophobic regions of protein C.

In this work we have characterized this region, using fluorescence spectroscopy techniques to assess membrane rupture, alteration of the fluorescence signal of FPE-labelled membranes in the presence of this peptide and steady state-fluorescence anisotropy. Calorimetric studies using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) were also performed, using several model membrane systems. This characterization was motivated by the fundamental role that this protein could play in the viral infection and the importance of this specific region in the interaction with biological membranes, an essential step of enveloped viruses' infection.

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P05-52
An infrared microspectroscopy 2DCOS study of the effect of radiation on normal and cancer cells

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Radiotherapy is widely used in cancer treatment, and there is a growing interest in knowing the effect of irradiation at cellular and molecular level. Infrared microspectroscopy combined with software techniques such as two-dimensional correlation spectroscopy (2DCOS) has the potential to offer an answer to the study of metabolic changes produced in cells subjected to irradiation. Keratinocyte cells from normal (HOK) or cancer (SCC25) cell lines have been subjected to different doses and 2DCOS maps have been obtained. The results are analysed either by looking at variations at a given radiation dose or the effect of different radiation doses on single cell lines. It is observed that at 100 cGy radiation, normal cells are more affected than cancer cells whereas at 200 cGy the changes induced by irradiation in cancer cells are different. Increasing the intensity of the irradiation dose does not change the pattern of the synchronous map in normal

cells, whereas in cancer cells high radiations doses produces maps compatible with no metabolic activity, a behaviour that has also been found in the TGase activity of the cells.

P05-53
Probing the structure-function relationship of [FeS] clusters and oligomeric assembly in [NiFe] hydrogenases

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[NiFe]-hydrogenases, metalloenzymes that produce or oxidise H_2, are important both for understanding the energy balance and metabolism of microoganisms as well as for serving as biological models for future hydrogen catalysts (1,2). These extremely efficient electrocatalysts have been studied using electrochemistry, EPR, crystallography and molecular biology.

A special sub-category of hydrogenases, known as oxygen tolerant hydrogenases, display sustained activity in the presence of oxygen and are therefore of particular interest for electrocatalyst design[1]. The crystal structures of 'standard' (O_2-inactivated) hydrogenases have been known for many years, but only recently have the structures of oxygen tolerant hydrogenases been resolved (3–5).

There is a tendency for membrane-bound hydrogenases to be oligomeric (5,6): for example the *E. coli* Hyd-1 is a dimer of heterodimers. Oligomer formation has important implications, not least in technological applications that require protein surface modification, but also perhaps in the function of these enzymes in the organism. We have therefore studied oligomer formation in Hyd-1 alongside activity measurements to establish whether any correlations exist.

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P05-54
All-trans retinoic acid modulates the expression and activity of mitochondrial oxidative phosphorylation complexes

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All-trans-retinoic acids (ATRA) is one of the biologically active metabolites of vitamin A which plays an important role in cell differentiation and proliferation (MB. Reeves *et al.* 2007, Science). The molecular basis of its action has not been fully elucidated. It was previously shown (F. Papa *et al.* 2007, Int J Immun Pharm) that ATRA treatment of normal human keratinocytes resulted in growth suppression, increase of complex I content and reduction of the NADH-UQ oxidoreductase enzymatic activity. These effects were associated with enhanced level of GRIM-19. We found that induction of cAMP-PKA signalling, by dibutyryl cyclic AMP or okadaic acid, restores the complex I activity inhibited by ATRA, indicating an interplay between ATRA and PKA signal transduction on regulation of cellular bioenergetics. Mitochondrial proteome has to be considered as a non-static entity, that shows characteristic changes according to the functional state of the cell. To monitor the effect of ATRA on mitochondria keratinocytes protein profile a wide-search proteomic approach was used. In ATRA treated cells, a large number of mitochondrial proteins, were found to be up or down expressed with respect to control cells. In particular ATRA cell-treatment appeared to affect proteins which are synthesized in very low amount, as the case of regulatory proteins.

Two proteins spots, down regulated in mitochondria of ATRA treated keratinocytes, were identified by mass spectrometry analysis as ATP synthase beta subunit, component of complex V, and protein disulfide-isomerase A6. These results indicate an effect of ATRA on the expression of mitochondrial OXPHOS complexes.

P06 – Cell Signaling

P06-1
Grainyhead-like 1 GRHL1 transcription factor in signaling pathways and in development of skin cancers

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GRH family of transcription factors is ancient and has appeared in evolution in early unicellular ancestor of animals, choanoflagellates, and fungi. In extant animals, as evolutionary distinct as insects and man, they are involved in regulation of development, functioning and regeneration of epidermal barrier and in other processes.

In mammals there are three factors – Grainyhead-like 1/2/3 (GRHL1/2/3). All of them are highly expressed in mice epidermis and appear to be crucial for the maintenance of this organ. Among them GRHL1 regulates expression of keratinocytes' cell junction component – desmosomal cadherin desmoglein 1 (DSG1).

There are numerous reports linking the GRHL transcription factors to carcinogenesis. Decreased GRHL2 expression inhibits the growth of hepatoma cells, suggesting its association with cell proliferation, and in the case of human oral SCC cells – its expression is increased. Moreover, recently a link between the GRHL3 gene and skin cancer has been identified in human patients as well as in a mouse model. Tissue-specific deletion of Grhl3 in mouse adult epidermis evokes loss of expression of PTEN, a direct GRHL3 target, resulting in aggressive squamous cell carcinoma (SCC) induced by the activation of PI3K/AKT/mTOR signaling.

The involvement of the GRHL1 transcription factor in signaling pathways and in carcinogenesis has not yet been studied. My preliminary experiments, performed using animal model, demonstrated increased susceptibility of Grhl1-deficient mice to chemically-induced skin carcinogenesis. I would like to present my results of my investigation on details of the role of GRHL1 transcription factor in development and progression of epidermal carcinomas.

P06-2
PARP-1 regulates Ets-1 transcriptional activity in cancer cells

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Ets-1 is the founding member of the Ets transcription factor family. Mainly involved in embryonic development, its over-expression is associated with a poor prognostic in invasive pathologies such as cancers. Ets-1 regulates gene expression by binding to specific DNA elements found in the promoters of its target genes, called Ets binding sites (EBS). Ets-1 functions are controlled by interaction with partners to select target genes, to modulate its ability to bind DNA and also to regulate its transcriptional activity. Therefore, the understanding of its role in cancer progression is linked to the characterization of its interaction partners. In order to identify novel interaction partners of Ets-1, we carried out a large scale strategy called streptavidin pull-down. Several potential partners were identified by MALDI-TOF mass spectrometry. Among those, we were able to identify, interacting with Ets-1, the Poly (ADP-Ribose) Polymerase-1 (PARP-1). PARP-1 is an abundant nuclear protein which catalyzes poly-ADP-ribosylation (PARylation) and plays diverse roles in many molecular and cellular processes, such as DNA damage detection and repair and chromatin modification. Recent studies have revealed that besides its role in DNA repair pathway, PARP-1 is involved in the regulation of different transcription factors including the Ets Family. In this study, we show that Ets-1 interacts directly with PARP-1 and is parylated in return. Using PARP-1 catalytic inhibitors, our results show that parylation of Ets-1 has direct consequences on its transcriptional activity. Taken together, these findings strengthen the idea of a functional link between Ets transcription factors and DNA repair proteins. Furthermore, inhibition of PARP-1 could be a new strategy to target Ets-1 activity in tumours.

P06-3
Dynamic regulation of microtubules orientation by Gibberellins and Prefoldin-complex

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Plant morphogenesis relies on specific patterns of cell division and expansion. For instance, shoot elongation is achieved through anisotropic cell growth. It is well established that cortical microtubules at the inner tangential wall of epidermal cells orient the direction of cell expansion through their influence on the deposition of cell wall material, but less is known about the molecular mechanisms that determine microtubule arrangement within the cells. Among the signals that regulate microtubule dynamics, the phytohormones gibberellins are unique in that they promote cell expansion and also direct the orientation of the cortical microtubule array perpendicular to the growth axis, raising the question of how these two processes are coordinated. DELLA proteins mediate transcriptional regulation by gibberellins of cell expansion genes. Here we show that DELLA proteins also modulate cortical microtubule orientation through the interaction with the prefoldin complex, a chaperone required for tubulin folding. We found that the interaction between DELLA and two subunits of prefoldin prompts the relocation of the complex into the nucleus, and that this localization is dependent on gibberellin signaling. As a consequence of the mislocalization of prefoldin, the availability of alpha/beta-tubulin heterodimers is severely compromised. The physiological relevance of this molecular mechanism was confirmed by the observation that the daily rhythm of plant growth was accompanied by coordinated oscillation of DELLA accumulation, prefoldin subcellular localization, and cortical microtubule reorientation.

P06r-4
Vasoactive intestinal peptide induces p53 activation in human clear cell renal cell carcinoma

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Incidence of renal cell carcinomas (RCC) has been increasing over the past three decades. The renal tumors are highly angiogenic and are resistant to conventional interventions, particularly

radiotherapy.Hence, we studied the effect of VIP on p53 and PCNA expression, intracellular ROS levels, the expression and secretion of vascular endothelial growth factor (VEGF) and nuclear levels of p50. Reverse transcriptase (RT)-polymerase chain reaction (PCR), immunocytochemistry, western blotting, enzyme immunoanalysis and ROS levels were performed. VIP down-regulated the expression of various downstream gene products that mediate cell proliferation (PCNA) and angiogenesis (VEGF), and induced reactive oxygen species (ROS). This fact could be due to increased the expression of p53. Overall, these findings suggest that VIP can interfere with multiple signaling cascades involved in tumorigenesis and used as a potential therapeutic candidate for both the prevention and treatment of cancer.

P06-5
Characterization the effects of a novel PKD1 inhibitor in VEGF signaling pathway on endothelial cells

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Familial frontotemporal lobar degeneration (FTLD-TDP) has been linked to mutations in progranulin gene (PGRN) that lead to progranulin haploinsufficency, although the pathogenic mechanism of PGRN deficit is largely unknown. Allelic loss of PGRN was previously shown to increase the activity of cyclin-dependent kinase (CDK) CDK6/pRb pathway in lymphoblasts expressing the c.709-1G > A PGRN mutation. Since members of the CDK family appears to play a role in neurodegenerative disorders and in apoptotic death of neurons subjected to various insults, we used established lymphoblastoid cell lines from control and carriers of c.709-1G > A PGRN mutation (asymptomatic and FTLD-TDP diagnosed individuals) to performed a comparative study of cell viability after serum withdrawal. Our results suggest that the CDK6/pRb pathway is enhanced in the c.709-1G > A bearing lymphoblasts. Apparently, this feature allows PGRN-deficient cells to escape from serum withdrawal-induced apoptosis by decreasing the activity of executive caspases, lowering the dissipation of mitochondrial membrane potential and the release of cytochrome c from the mitochondia. Inhibitors of CDK6 expression levels like sodium butyrate or the CDK6 activity such as PD332991 were able to restore the vulnerability of lymphoblasts from FTLD-TDP patients to trophic factor withdrawal. The use of PGRN-deficient lymphoblasts from FTLD-TDP patients may be a useful model to investigate cell biochemical aspects of this disease. It is suggested that CDK6 could be potentially a therapeutic target for the treatment of the FTLD-TDP.

P06-6
Collagen-sericin 3D scaffold enhances adipogenic differentiation of hADSCs

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Natural compounds are currently required for improvement of tissue engineering strategies. This study focuses on human adipose-derived stem cells (hADSCs) adipogenic differentiation potential in a novel biodegradable and biocompatible collagen-sericin 3D scaffold (Coll-Ser-H), compared to control collagen hydrogel (Coll-H).

hADSCs in the 4th passage were seeded at a density of 2×10^5cells/cm^2 on Coll-H and Coll-Ser-H surfaces. However, cells populated deeper layers of the scaffold, resulting in 3D systems, which were exposed to adipogenic induction cocktail for 28 days. RealTime PCR and flow citometry assays were performed at 3,7,14,21 and 28 days to investigate adipogenic markers PPARγ2 and aP2 expression during differentiation process. SEM (Scanning Electron Microscopy) studies contributed on understanding hADSCs' distribution and shape evolution inside the scaffolds towards the mature adipocytes.

OilRedO highlighted intracellular lipid droplet accumulation, while SEM revealed distinct stages in adipocyte growth in Coll-Ser-H system compared to control at 7,14 and 21 days of adipogenesis. Late adipogenic marker aP2 displayed increased levels of expression in Coll-Ser-H than in Coll-H after 7 days of differentiation, while PPARγ2 acted as the inductor of adipogenesis in both systems. Flow citometry results confirmed these observations at a proteic level of expression.

Upregulated adipogenic pattern suggests that Coll-Ser-H could be a suitable 3D culture system for soft tissue reconstruction due to its good biocompatibility (data not shown) and capacity to support hADSCs' differentiation. However, Coll-Ser-H inflammatory properties should be addressed in further work.

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P06-7
Hedgehog/GLI and IL6/STAT3 signal cooperation in basal cell carcinoma

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The Hedgehog (HH) pathway is a key signaling system during vertebrate development and controls multiple aspects of tissue homeostasis in adult organisms. In line with its critical function in proliferation, survival and stem cell fate, inappropriate activation of HH/GLI signaling is involved in a number of human malignancies including basal cell carcinoma (BCC), the most common cancer in the western world.

The activity of HH signaling can be modulated by signals such as RAS, PI3K, or EGFR/AP1, resulting in increased oncogenic HH/GLI activity. In a focused small-scale screen with the aim to identify novel cooperative signals synergizing with the HH/GLI in oncogenic transformation we identified the Interleukin-6/Signal Transducer and Activator of Transcription 3 (IL6/STAT3) cascade as potent enhancer of oncogenic HH/GLI signaling. Concomitant activation of HH/GLI and IL6/STAT3 resulted in transformation of human non-tumorigenic keratinocytes, while neither signal alone was able to elicit this phenotype. Human and mouse BCC lesions displayed high levels of active STAT3 protein and other key effectors of the IL6 pathway. Furthermore, pharmacological inhibition as well as RNAi mediated knockdown of STAT3 in a HH dependent BCC cell line demonstrated the crucial role of the STAT3 transcription factor for HH dependent cellular transformation *in-vitro* and *in-vivo*. Our data identify a novel positive modulator of HH/GLI signaling in cancer and suggest that simultaneous inhibition of HH/GLI and IL6/STAT3 signaling may constitute a promising combination therapy for HH/GLI dependent cancers.

P06-8
The opposite role of glucocorticoid and alpha1- adrenergic receptors in stress triggered apoptosis of rat Leydig cells

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This study was designed to evaluate and define the mechanism of the stress-induced pro/anti-apoptotic signaling in Leydig cells using the three *in vivo* models: (1) acute/repeated immobilization stress (IMO); (2) intratesticular blockade of glucocorticoid receptors (GRs) before/under IMO; (3) intratesticular blockade of α1-adrenergic receptors (α1-ADRs) before/under IMO. Results showed that IMO applied once (1xIMO) or twice (2xIMO) increased apoptosis of Leydig cells, while after 10xIMO the number of apoptotic cells back to the control level. The reduced oxygen consumption, and the mitochondrial membrane potential, liking of cytochrome c from mitochondria, increased level of caspase-9 followed by increased caspase-3 activity were registered in Leydig cells obtained from 1xIMO or 2xIMO rats, but back to control levels after 10xIMO. In Leydig cells from rats exposed to 10xIMO, increase in Igf2/Igf2r transcripts, and anti-apoptotic recovery kinases (ERK, total AKT) proteins, as well as pAKT/AKT ratio in mitochondria was detected. In this signaling scenario, the transcriptional milieu of α1-ADRs and Hsd11b2 in Leydig cells were up-regulated, while Gr transcription was not affected by IMO. Blockade of intratesticular GRs diminished the pro-apoptotic effects of 1xIMO or 2xIMO and 10xIMO-induced Hsd11b2 increase, but didn't changed 10xIMO anti-apoptotic effects. Blockade of intratesticular α1-ADRs diminished the anti-apoptotic effects of 10xIMO, but didn't changed 1xIMO or 2xIMO pro-apoptotic effects and 10xIMO-induced Hsd11b2 increase. Accordingly, data pointed a critical role of glucocorticoids into mitochondria-targeted apoptosis induction and it is first report showing the possible engagement of α1-ADRs in formation of Leydig cell apoptotic resistance to prolonged stress.

P06r-9
G protein-coupled receptor kinase 2 (GRK2) contributes to cellular transformation and breast tumor progression

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G protein-coupled receptor kinase 2 (GRK2) is emerging as a key, integrative node in many signalling pathways. Besides its canonical role in the modulation of the signaling mediated by G protein-coupled receptors (GPCR), this protein can display a very complex network of functional interactions with a variety of signal transduction partners, contributing to the proper functioning of basic cellular processes such as cell migration or cell cycle progression. Consequently, both expression levels and activity of GRK2 are tightly controlled in normal settings but frequently unbalanced in several relevant pathological contexts like inflammatory, metabolic or cardiovascular diseases and in some tumors, thus suggesting that such changes may be involved in the onset or development of those pathologies. In this context, we find that GRK2 is up-regulated in human transformed epithelial mammary cell lines as well as in the mammary gland of murine models that are prone to develop breast tumors. Interestingly, such up-regulation of GRK2 relies on the over-activation of signaling pathways triggered by ErbB2 receptors or by estrogen receptors in those hormone-responsive breast tumor cell lines. Moreover, accumulation of GRK2 protein seems to contribute to cellular transformation by enhancing both mitogenic and antiapoptotic activities of relevant stimuli in breast tumor progression such as heregulin. On the contrary, GRK2 knock-down in breast cancer cell lines results in suppression of cellular growth both '*in vitro*' and '*in vivo*' models of cancer. Furthermore, GRK2 levels are increased in a very significant proportion of infiltrating ductal carcinoma samples from patients, strongly suggesting that GRK2 is a relevant modulator of tumor survival and progression.

P06-10
What is the reason for the deregulation of the STAT signaling pathway in malignant pleural mesothelioma?

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Background: STAT1 and STAT3 are dysregulated in human malignant pleural mesothelioma (MPM), an aggressive cancer with little knowledge about predictive factors of outcome. The negative feedback-loop of STAT1 is not functioning: SOCS1 and PIAS1 are downregulated. MicroRNAs (miRNAs) regulate the expression of target mRNAs. Therefore we aimed to quantify selected miRNAs in MPM which are thought to be involved in the regulation of the STAT signaling pathway. Methods: RNA was obtained from 35 formalin-fixed and paraffin-embedded tumor tissue samples. MiRNAs were selected via *in silico* target prediction tools. Quantitative real-time PCR was used to assess miRNA expression levels. An immunohistochemical (IHC) staining with nine antibodies was performed on tissue microarray sections to correlate it with the results of the miRNA detection.
Results: MiR-106a (targeting STAT3) expression was increased in 63% of cases. MiR-155, miR-19a and miR-30d* (targeting SOCS1, SOCS1, STAT1, respectively) were downregulated in all cases. Due to very low expression levels, miR-196a*, miR-608 and miR-765 (targeting SOCS6, PIAS1, SOCS3 respectively) were not detected. Positive IHC staining was achieved for STAT1, pSTAT1(Ser727), STAT3, PIAS1, PIAS3, Sos1 and OSM. STAT1 was higher expressed than STAT3; SOCS1 and SOCS3 were not detected by IHC. Conclusion: The inverse correlation between pSTAT1 and miR-30d* ($p = 0.014$) indicates a regulatory effect and this miRNA may interact with STAT1 ($p = 0.062$). Neither STAT3 nor Sos1 and OSM are affected by miR-106a ($p = 0.53$, $p = 0.29$, $p = 0.8$ respectively) although this miRNA is expected to play an important role in MPM. One part of the negative feedback-loop is totally missing: SOCS1 and SOCS3 were not detected.

P06-11
Vasoactive intestinal peptide (VIP) suppresses the metastasis potential of human clear cell renal cell carcinoma

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The molecular mechanisms involved in the development and progression of clear cell renal cell carcinomas (ccRCCs) are poorly understood. A common genetic mutation found in ccRCC is the loss of the von Hippel-Lindau (VHL) gene, which results in stabilization of hypoxia-inducible factors (HIFs), and contributes to cancer progression and metastasis. The present study was undertaken to investigate the VIP effect on metastatic factors in human VHL-null A498 ccRCC and HK2 renal cells. Gelatin zymography together with cell adhesion and invasion assays were performed. VIP increased cell adhesion in A498 cells but not in HK2 cells. Furthermore, the expression levels of metaloproteinases MMP2 and MMP9, tumoral cell migration were decreased after VIP treatment in A498 cells but not in HK2 cells. This pattern of anti-metastatic effects of VIP was blocked by the specific VIP antagonist JV-1-53, supporting the direct involvement of VIP receptors in the observed responses. In conclusion, these data suggest that VIP has an important role in preventing invasion and metastasis in A498 cells, and could be a potential therapeutic target in ccRCCs.

P06-12
Presynaptic kainate receptor-mediated facilitation of glutamate release involves calcium-calmodulin at mossy fiber-CA3 synapses

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Presynaptic kainate receptors (KARs) modulate the release of glutamate at synapses established between mossy fibers (MF) and CA3 pyramidal neurons in the hippocampus. The activation of KAR by low, (nanomolar), kainate (KA) concentrations facilitates glutamate release. KAR-mediated facilitation of glutamate release involves the activation of an adenylate cyclase/cyclic adenosine monophosphate/protein kinase A (AC/cAMP/PKA) cascade at MF-CA3 synapses. Here, we studied the mechanisms by which KAR activation produces this facilitation of glutamate release. We have found that the facilitation of glutamate release mediated by KAR-activation requires an increase of Ca^{2+} levels in the cytosol and the formation of a Ca^{2+}-calmodulin complex to activate AC. The increase in cytosolic Ca^{2+} underpinning this modulation is achieved, both, by Ca^{2+} entering via Ca^{2+} permeable KARs and, by the mobilization of intraterminal Ca^{2+} stores. Finally, we have found that, congruent with the Ca^{2+}-calmodulin support of KAR mediated facilitation of glutamate release, induction of LTP at MF-CA3 synapses has an obligate requirement for Ca^{2+}-calmodulin activity.

P06-13
C3G transgenic models in platelets reveal a role for C3G in platelet function and chronic myeloid leukemia development

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We have generated mouse transgenic lineages for C3G and C3GΔCat (C3G mutant lacking the GEF domain), where the transgenes are expressed under the control of the megakaryocyte and platelet specific PF4 gene promoter. Transgenic platelet activity has been analyzed through *in vivo* and *in vitro* approaches, including bleeding time, aggregation assays and flow citometry. Transgenic C3G animals showed bleeding times significantly shorter than control animals, while tgC3GΔCat mice presented a remarkable bleeding diathesis as compared to their control siblings. Accordingly, platelets from tgC3G mice showed stronger activation and aggregation in response to platelet agonists such as thrombin, PMA, ADP or collagen than control platelets, while those from tgC3GΔCat animals had a lower response. In addition, we present data indicating that C3G is a mediator in the PKC pathway leading to Rap1 activation. Remarkably, a significant percentage of tgC3G mice presented higher levels of neutrophils than their control siblings. This unexpected result prompted us to investigate whether our transgenic model would be useful to analyze the role of C3G in chronic myeloid leukemia (CML), previously suggested by several reports. The Bcr-Abl transgenic model used develops physiopathological characteristics of CML in less than 10 months, including granulocytic sarcoma of the lung, indicative of extramedullar blast crisis. Double Bcr-Abl/C3GΔCat transgenic mice present lower neutrophil percentages, non-pathological WBC counts, less splenomegaly and less myeloid cell infiltrates, as compared to single Bcr-Abl mice. These results indicate that C3G plays a role in platelet clotting through mechanisms involving its GEF activity, and suggest that it might be also involved in the development of CML.

P06r-14
Transactivation of HER-2 by growth hormone-releasing hormone (GHRH) in human androgen-independent prostate cancer cells. Effect of the GHRH antagonist, JMR-132

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The growth hormone-releasing hormone (GHRH) acts an autocrine growth factor in prostate cancer. GHRH could be involved in the progression of prostate cancer and androgen independence as well as be related to poor prognosis. Tyrosine kinase receptor HER-2 is associated with the development of androgen independence in prostate cancer. In this study, we evaluated the transactivation of HER-2 by GHRH and the effects of the GHRH antagonist, JMR-132 on HER-2 activation in human androgen-independent prostate cancer PC3 cells. Western blot assays showed an increase of phosphorylated HER-2 by GHRH in a time-dependent manner with the highest responses at 30 sec and 30 min. Thereafter, we assessed the involvement of metaloproteinases (MMP)-2 and 9, ADAMs, p-SRC and PKA in such a transactivation using specific inhibitors. Our results demonstrated that the early activation was mediated by p-Src and PKA whereas MMPs and ADAMs were involved in the late activation. These effects were blocked when we incubated PC3 cells with the GHRH antagonist, JMR-132. Finally, we analyzed the effect of JMR-132 on HER-2 expression levels in an *in vivo* experimental model. For this purpose, tumor cells were injected in nude mice to obtain PC3 xenografts. After treatment with JMR-132 (10 μg/day), we observed a decrease of tumor weight as well as decreased expression levels of both HER-2 mRNA and phosphorylated protein as compared with control group. These findings shed light on the mechanisms of action of GHRH and the inhibitory effect of its antagonist in human androgen-independent prostate cancer.

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P06r-15
A new phosphorylation/acetylation switch in the regulation of cortactin and its role in cell spreading

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Cortactin is a multidomain protein that plays a central role in the remodeling of the actin cytoskeleton in virtue of its capacity to active the Arp2/3 complex and N-WASP protein[1]. Cortactin is an oncoprotein overexpressed in different human carcinomas and currently considered a *bona fide* invadopodia marker. In addition, cortactin is targeted by pathogens to invade or to adhere to the host cells[2]. Although cortactin was cloned as a Src substrate how this post-translational modification regulates the activity of the protein remains unclear. Moreover the protein is regulated by acetylation but how these two post-translational modifications relate to each other was unknown. Therefore to analyze the biochemical consequences of cortactin phosphorylation on tyrosines, we used a fusion expression system that forces the interaction between cortactin and Src kinase in cells. We describe here a new regulatory mechanism: a competition between the acetylation and the tyrosine-phosphorylation of cortactin. This switch was confirmed with the endogenous protein. Furthermore, we analyzed the effect of the phosphorylation in cortactin-Focal Adhesion Kinase (FAK) complex formation during cell spreading[3]. Finally we present new unpublished data of how the cortactin-FAK complex relates to N-WASP protein.

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P06-16
Development of an internalisation assay for G Protein Coupled Receptors and Receptor Tyrosine Kinases using Bioluminescence Resonance Energy Transfer (BRET) technology

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Internalisation and subsequent degradation of receptors located at the plasmamembrane represent a desired therapeutic approach to eliminate those receptors whose activation leads to unwanted cellular behavior; typical examples include aberrant proliferation or mobility in the field of oncology. For example, the application of therapeutic antibodies against relevant receptors is based on such internalisation/degradation mechanisms. In order to follow the trafficking of endocytosed receptors and thus the efficacy of agents directing these receptors into the internalisation/degradation pathway, markers for their localisation at the plasmamembrane (K-Ras), early (Rab5, EEA1) and late (Rab7) endosomes, and lysosomes (Rab7, Lamp2) have been described previously. We reasoned that such markers could be used in BRET assays (live cells) to time-dependently follow the destination of internalised receptors and quantify the efficacy of agents that force the receptors into lysosomes, as BRET measures relative distances between proteins. We therefore started with GPCRs, by coexpressing the Renilla luciferase-tagged D2 dopamine receptor (D2-R), with GFP2-tagged K-Ras, Rab5 or Rab7 in CHO cells. We observed a basal BRET2 signal between D2-R and K-Ras that was robustly diminished by a 1 hour agonist treatment (1 μM dopamine). This signal was completely antagonized by 10 μM haloperidol treatment. At the same time point the BRET2 signals between D2-R/Rab5 and D2-R/Rab7 were enhanced by the agonist and again neutralized by the antagonist. Using this technology we were thus able to follow the trafficking of the D2-R from the plasmamembrane to the endosomal/lysosomal compartments. Other types of receptors, such as Receptor Tyrosine Kinases are also being tested now, to see whether this approach may be extended to other classes of endocytosed receptors.

P06-17
Ceramide 1-phosphate induces CD69 expression in immune cells through a mechanism involving ERK1-2 activation

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The bioactive sphingolipid ceramide-1-phosphate (C1P) regulates vital cellular functions in macrophages. These include regulation of cell proliferation, apoptosis and cell migration. In the present work, we show that C1P can induce the expression of the lectin-like receptor CD69, very early activation marker, in a variety of immune cells including various types of lymphocytes (T-cells, B-cells, natural killer cells) and myeloid cells (e.g. neutrophils, eosinophils, monocyte/macrophages), indicative of immune cell stimulation.

We also show that C1P stimulates phosphorylation of the extracellularly regulated kinases (ERK)1-2 and the transcription factor NF-kB in a mixed population of immune cells (spleen derived mononuclear cells). Consistently with previous works, we found that NF-kB lies downstream of ERK1-2 in this pathway. Noteworthy, inhibition of mitogen-activated protein kinase kinase (MEK), the enzyme that phosphorylates and activates ERK1-2, completely blocked C1P-induced CD69 expression, suggesting that the MEK/ERK1-2 pathway is essential in this process. However, NF-kB does not seem to be involved as inhibition of the activity of this transcription factor had no effect in the stimulation of CD69 expression by C1P. Therefore, it can be concluded that C1P induces CD69 expression in manner that is dependent upon prior activation of the MEK/ERK1-2 pathway but independent of NF-kB stimulation.

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P06-18
Human 825C>T polymorphism in GNB3 gene promotes enhanced cell migration by inducing cytosolic calcium influx and hyper phosphorylation of ERK regulated mTOR pathway

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The common human 825C > T polymorphism in the GNB3 gene results in smaller and stable Gβ3s protein subunit, that has been implicated in causing hypertension and obesity. A previous cohort study in cancer patients carrying GNB3 825C > T polymorphism reported to have different biological behaviour of tumour disease leading to significant higher risk both for tumour relapse and death. The dissected signalling mechanism contributing to enhanced cell migration phenomenon in the presence of the Gβ3s subunit has not been fully elucidated. In our study, EBV transformed lymphoblast cells expressing Gβ3s subunit revealed enhanced chemotactic migration kinetics upon treatment with various chemo-attractants such as VEGF, EGF, and TGFβ. An increase in cytosolic Ca^{2+} ion influx activating ERK phosphorylation (pERK) but not Akt1 (pAkt1), is observed lymphoblast cells expressing Gβ3s subunit in comparison to normal lymphoblast cell both at basal and EGF stimulated conditions. Despite the loss in pAkt1, a remarkable increase in phosphorylation of FoxO3a, mTOR, $p70^{S6K}$ and 4E-BP1 is observed in lymphoblast cells expressing Gβ3s subunit. COS-7 stable clones expressing Gβ3s subunit showed enhanced wound healing when compared to cell expressing normal Gβ3 subunit, indicating that enhanced migration observed in the presence of Gβ3s subunit is not cell dependent phenomenon. Our results clearly indicate that Gβ3s subunit exerts enhanced cytosolic Ca^{2+} ion influx to hyper activate ERK and mTOR pathway substrates to enhance cell migration. Therefore this atypical regulation of Ca^{2+} > ERk > mTOR pathway in the presence of Gβ3s subunit might be responsible for enhanced tumour relapse and increased metastasis in cancer patients carrying GNB3 825C > T allele.

P06-19
Evidences of a cGMP signaling pathway in the primitive aquatic fungus *Blastocladiella emersonii*

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Cyclic GMP is a key signaling molecule that regulates a wide array of cell processes. In contrast to the lack of evidence of a cGMP pathway in published genomes of ascomycete and basidiomycete fungi, previous studies have shown changes in the levels of cGMP during the life cycle of the blastocladiomycete *Blastocladiella emersonii*. Evolutionists attribute the absence of cGMP signaling pathways in higher fungi to the sedentary life style of these organisms. However, the blastocladiomycete class of fungi has motile cells in at least one stage of their life cycle, which could explain the existence of this pathway in these primitive fungi. Thus, the purpose of this study is to confirm the existence and characterize the cGMP signaling pathway in this fungus. Sequences obtained by 454 sequencing and 5′RACE experiments revealed the complete nucleotide sequence of three putative guanylate cyclases (BeGC1, BeGC2 and BeGC33) and a cGMP phosphodiesterase (BePDE), and in addition, the presence of a putative cGMP-activated potassium channel. The identification of the function of these GCs are been carried out. BeGC3 appears to be composed of an N-terminal heme-PAS domain and a guanylyl cyclase domain, probably functioning as a NO-regulated cyclase. The BeGCs and the potassium channel genes have been cloned and recombinant proteins were expressed in *E. coli* for further antibody production and intracellular localization. Comparative sequence analysis and modeling of BePDE indicate that amino acid residues important for catalysis and substrate coordination are conserved, confirming its identity as a cGMP phosphodiesterase. Recombinant BePDE produced in *E. coli* presents activity towards hydrolysis of cGMP, supporting that this enzyme is active in the fungus. All these data indicate that cGMP signaling is present in *B. emersonii* and these new enzymes involved in this pathway are been characterized.

P06-20
Degradation of GRK2 is cell cycle regulated by Mdm2 and APC/C ubiquitin ligases

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Cell cycle progression requires changes in the activity or levels of a variety of key signalling proteins. Timely proteasome-dependent degradation of key kinases that are ubiquitinated by diverse E3 ligases such as Mdm2 (Murine Double Minute 2) or APC/C (Anaphase Promoting Complex/Cyclosome) represents a major regulatory mechanism in the control of cell cycle. We have recently reported that GRK2, a serine/threonine G protein-coupled receptor kinase, is part of an intrinsic pathway that ensures timely progression of cell cycle at G2/M. Although initially identified as a key player in the desensitization and internalization of manifold GPCR receptors, GRK2 must be considered as a signal mediator due to its ability to phosphorylate/interact with a variety of signaling proteins and perform cell functions. We have found that GRK2 protein levels progressively decay during G2 as a result of its functional interaction with CDK2/cyclinA and Pin1, which ultimately trigger the Mdm2-dependent ubiquitination of GRK2. In addition to this degradation pathway, the decay of GRK2 is actively maintained at mitosis onset by the APC/C ligase complex. We have identified a D-Box-like degron in GRK2 that is recognized by a subpopulation of active APC/C^{Cdc20} in a spindle checkpoint-independent manner. Finally, we provide evidences that such D-box motif might be modified to prevent GRK2 degradation by APC/ C^{Cdh1} in order to ensure the rapid recovery of GRK2 levels during G1-phase. Overall, sequential cooperation of Mdm2 and APC/C activities are critical to promote the default down-modulation of GRK2 in order to assure normal cell cycle progression.

P06-21
The presence of phosducin in thyroid parafollicular C cells

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Phosducin is one of the G protein-dependent signal transduction pathway regulatory proteins. In retina and pineal gland dephosphorylated form of this 33 kDa protein interacts with many points of the β subunit surface making impossible to heterotrimer assembling. In this way impairs the transduction of signal generated by the receptor. There are reports on the phosducin expression in some other tissues as well. In thyroid many G protein dependent pathways are active, the major is connected with activation of TSH receptor in follicular cells. The parafollicular C cells, the second type of endocrine cells, belong to the diffused neuroendocrine regulatory system and are present in thyroid in several times smaller amount than follicular cells. In these cells the G proteins dependent signal transduction pathways also exists. In the present work we have studied the presence of phosducin in thyroid. The experiments were performed on thyroid follicular cells (Nthy-ori 3-1 cell line), parafollicular C cells (TT cell line) as well as thyroid postoperative tissues from patients with struma nodosa. To detect phosducin in purified samples two approaches were used. The expression of phosducin gene was investigated by PCR followed by its product sequence analysis. The presence the protein form of phosducin was tested by electrophoretical methods. Electroseparated 33 kDa protein band was concentrated by electroelution, and after the second electrophoresis followed by immunoblotting, phosducin was visualized using three polyclonal anti-phosducin antibodies ap33, ap34 and ap36. We found that phosducin is present in thyroid gland and its expression occurs only in parafollicular C cells but not in the main follicular thyroid cells. The small quantity of parafollicular cells in thyroid results in very small amount of this protein in the tissue.

P06-22
Role of Ceruloplasmin in silica-induced changes of PI3K signal transduction pathway and the epigenetic modifications

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Silica is one of the most serious occupational hazard factors which can lead to lung fibrosis after long-term inhalation. Ceruloplasmin is an importment protein factors which is closely related to pulmonary fibrosis. Previous studies had shown that Cp could promot silica-induced increase of cell proliferation through JNK-c-Jun/c-Fos and ERK-c-Fos signaling pathways. Human embryonic lung fibroblasts were used to explore the changes of PI3K-Akt signal transduction pathway and the Histone acetylation. Studies showed that Silica can induce protein lysine acetylation increased. There were no significant effects of total lysine acetylation levels which were induced by Cp, but Cp could significantly reversed silica-induced increase in the acetylation of lysine. Silica could induce histone H2B (lys5/12), H3 (lys9/14), H4 (lys12) acetylation increase. At the same time, silica induced histone H3 acetylation increased, and the site 2 methylation was reduced. PTEN as an inhibitor of the PI3K-Akt signal transduction pathway, inhibiting its expression could increase the extent of histone acetylation, indicating that the PI3K-Akt signal transduction pathway involved in histone acetylation. Results demonstrate that silica can induce the increase in histone acetylation, and reduction of histone H3 methylation, in order to initiate gene transcription, increase the amount of the corresponding protein. Is the role that Cp can reduce silica-induced histone acetylation the protective effect of OR damage?

P06r-23
Phosphatidylinositol (4,5)-bisphosphate depletion in Saccharomyces cerevisiae activates the Pkc1-mediated cell wall integrity MAP kinase pathway from endosomal compartments

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Class I phosphatidylinositol 3-kinases catalyze the conversion of PtdIns-4,5-P_2 into PtdsIns-3,4,5-P_3. In mammalian cells, this second messenger controls important functions, like cellular proliferation and inhibition of apoptosis; in fact the hyperactivation of this protein is commonly observed in cancer. The model yeast *Saccharomyces cerevisiae* constitutively lacks of class I PI3K activity. Heterologous expression of hyperactive versions of this protein in yeast leads to the growth inhibition due to the depletion of the essential plasma membrane pool of PtdIns-4,5-P_2 (Rodríguez-Escudero I. *et al*., 2005). This yeast model has proven to be useful for applied purposes such as the screening of PI3K inhibitors, but also provides a way to study the roles of PtdIns-4,5-P_2 in the yeast cell. We have performed a global tran-

scriptomic analysis upon PI3K expression in *S. cerevisiae* and found a pattern reminiscent of that of cell wall stress conditions. Consistently, PI3K but not a kinase-dead mutant version triggered the phosphorylation of the cell wall integrity (CWI) MAPK, as well as the expression of a typical CWI transcriptional reporter. Time-course analyses revealed that the loss of PtdIns-4,5-P_2 from the plasma membrane correlates with both MAPK activation and actin depolarization. Also, a retard in the internalization of the vital endocytic marker FM4-64 was observed. Pkc1, the yeast orthologue of mammalian protein kinase C upstream CWI pathway, was abnormally located in intracellular compartments that seemed associated to endosomes. We propose that loss of essential cortical phosphoinositides is sensed by the CWI pathway at endosomal compartments.

P06r-24
Characterization of brain-specific PDK1 conditional knock-in mice expressing the L155E mutation in the nervous system

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The phosphoinositide 3-kinase signaling pathway plays important roles in the central nervous system by mediating the effects of neurotrophic factors, neurotransmitters and hormones promoting neuronal survival, neuritogenesis and plasticity.

PDK1 is a crucial master kinase that, in response to PI 3-kinase activation, switches on a number of AGC-kinase family members including PKB/Akt. Activation of PKB by PDK1 relies on the interaction of the PH domains present on both kinases with PtdIns(3,4,5)P3, the PI 3-kinase product. By contrast, in order to activate the rest of targets including S6K, RSK and SGK, PDK1 interacts with a phosphorylated hydrophobic motif on those substrates through the PIF-pocket docking site.

To dissect the contribution of the different PDK1 targets to PI 3-kinase actions, two knock-in mice expressing two specific single-aminoacid mutations of PDK1 were previously generated. The PDK1 K465E impairing the binding of the PH domain to PtdIns(3,4,5)P3, and the PDK1 L155E that disrupts the PIF-pocket.

Since the PDK1 L155E mutation caused embryonic lethality, and to define *in vivo* the function of the PDK1 PIF-pocket dependent substrates in the central nervous system, we employed conditional knock-in strategies to direct the expression of the L155E mutant to neuronal tissues. Activation of PKB by BDNF reached normal levels in the PDK1 L155E primary cortical neurons, while activation of S6K and RSK was totally abolished. Phosphorylation of NDRG1, a specific SGK1 substrate, was only marginally affected. As a consequence, BDNF-mediated neuronal survival decreased, and apoptosis induced by serum withdrawal increased, in the PDK1 mutant cortical cultures when compared with control littermates. By contrast, in mice expressing the complementary PDK1 K465E mutation, neuronal viability was not compromised, thereby suggesting a prominent role of the PIF-pocket branch of the PDK1 pathway in controlling neuronal survival.

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P06-25
Ceramide-1-phosphate stimulates carbohydrate metabolism

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Ceramide-1-phosphate (C1P) is a bioactive sphingolipid that is implicated in the regulation of cell growth and survival, as well as in the control of cell migration. All of these actions require high energy levels to be available in the cells. We hypothesized that C1P might stimulate glucose uptake and metabolism so that cells could generate sufficient ATP to accomplish these vital biological functions. Here we show that C1P stimulates the incorporation of glucose by macrophages and that it enhances the production of ATP. C1P-stimulated glucose transport involved the glucose transporter GLUT3 and was insulin-independent. Investigation into the mechanism by which C1P exerts this action revealed that the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt) is a major pathway involved in this process. In addition, we observed that C1P-stimuated glucose uptake and metabolism were inhibited by pertussis toxin, a potent Gi protein inhibitor that is commonly used to block cell signaling through activation of Gi protein-coupled receptors. Taken together, these results demonstrate that C1P-stimulated glucose uptake is mediated by GLUT3 and is dependent upon activation of the PI3K/PKB pathway. This action may also involve the interaction of C1P with a putative plasma membrane receptor that is coupled to Gi proteins in macrophage plasma membranes.

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P06r-26
Inactivation of CDK/pRb pathway normalizes survival pattern of lymphoblasts expressing the FTLD-progranulin mutation c.709-1G>A

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Familial frontotemporal lobar degeneration (FTLD-TDP) has been linked to mutations in progranulin gene (PGRN) that lead to progranulin haploinsufficency, although the pathogenic mechanism of PGRN deficit is largely unknown. Allelic loss of PGRN was previously shown to increase the activity of cyclin-dependent kinase (CDK) CDK6/pRb pathway in lymphoblasts expressing the c.709-1G > A PGRN mutation. Since members of the CDK family appears to play a role in neurodegenerative disorders and in apoptotic death of neurons subjected to various insults, we used established lymphoblastoid cell lines from control and carriers of c.709-1G > A PGRN mutation (asymptomatic and FTLD-TDP diagnosed individuals) to performed a comparative study of cell viability after serum withdrawal. Our results suggest that the CDK6/pRb pathway is enhanced in the c.709-1G > A bearing lymphoblasts. Apparently, this feature allows PGRN-deficient cells to escape from serum withdrawal-induced apoptosis by decreasing the activity of executive caspases, lowering the dissipation of mitochondrial membrane potential and the release of

cytochrome c from the mitochondia. Inhibitors of CDK6 expression levels like sodium butyrate or the CDK6 activity such as PD332991 were able to restore the vulnerability of lymphoblasts from FTLD-TDP patients to trophic factor withdrawal. The use of PGRN-deficient lymphoblasts from FTLD-TDP patients may be a useful model to investigate cell biochemical aspects of this disease. It is suggested that CDK6 could be potentially a therapeutic target for the treatment of the FTLD-TDP.

P06r-27
Downregulation of ERK1/2 activity by CaMKII modulates p21 levels and survival of immortalized lymphocytes from Alzheimer's disease patients

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We have previously reported that lymphoblasts from Alzheimer's disease (AD) patients were more resistant to serum withdrawal-induced apoptosis than cells from age-matched controls in a Ca2+/CaM-dependent manner. These features were accompanied by downregulation of ERK1/2 activity and increased levels of p21 protein in AD cells compared with control cells. The aim of the present investigation was to delineate the molecular mechanisms underlying the distinct regulation of p21 content in AD cells. Quantitative RT-PCR showed increased p21 mRNA levels in AD cells, while the half-life of the protein remained unchanged. Inhibition of ERK pathway with PD98059 prevented the serum deprivation-induced death of control cells, increased both the p21 mRNA and protein levels and the nuclear localization of FOXO3A transcription factor, which was found to regulate p21 expression in our cells. In contrast, treatment of cells with the PI3K/Akt inhibitor Ly29004 had little effect on p21 levels and did not affect the survival of either control or AD cells. The CaM antagonist CMZ and the CaMKII inhibitor KN-62, were able to overcome the resistance to death of AD lymphoblasts by increasing the ERK1/2 activation, which in turn inhibited the transcription of p21 by postranslational inactivation of the transcription factor FOXO3A. Taken together, these data suggest that the decrease of ERK activity plays a role in the serum withdrawal-induced up-regulation of p21 and survival in AD lymphoblasts.

P06-28
Ochratoxin A induces autophagy on human colon cancer cell lines HCT116

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Ochratoxin A (OTA) is an important food-borne mycotoxin and occur in a wide range of food commodities. This mycotoxin has been shown to cause diverse toxic effects in animals and is also suspected of disease causation in humans. In the present study, we investigated the molecular mechanisms of OTA toxicity in human colon cancer cell lines (HCT116). To this aim, we have monitored the effect of OTA on the expression of several proteins involved in different cell death pathways namely apoptosis,necrosis and autophagy. Our results clearly showed that OTA inhibits cell proliferation in a dose-dependent manner on HCT116 cells. Treatment of cells with necrosis inhibitors (necrostatine) or apoptotic inhibitor (Zvad) failed to block cell death induced by OTA. Moreover, the treatement of HCT116 overexpressed Flip or Bax-defficient HCT116 cells with OTA does not inhibit OTA toxicity when compared to HCT116 treated cells. These findings prompted us to investigate the autophagic pathway. We found that caspase 3 and 8 are not activated in cells treated with OTA, therefore they are not involved in the signaling pathway induced by OTA.This mycotoxin induced the phosphorylation of p53 and the activation of caspase 2, 6 and caspase 10. It induced also the lamine, PARP and BID cleavage witch is not suppressed by the apoptotic inhibitor Zvad. Furthermore, OTA induced the conversion of LC3 I to LC3 II witch is an autophagosome marker. Altogether, our results suggested that, the HCT116 death caused by OTA could be mediated by the autophagic pathway.

P06-29
Study of the gene expression profile of breast cancer cells in the absence of PKCα

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PKCα is over-expressed in a wide variety of breast cancer cells. It has been associated with malignant transformation by increasing the proliferation rates, motility and invasiveness of cancer cells. Here we demonstrate that inhibition of PKCα expression in MCF-7 and MDA-MB-231 breast cancer cell lines reduces their proliferation rate. However, down-regulation of PKCα only affected migration of MCF-7 and invasion of MDA-MB-231 cells. Next step in our investigation was to compare the gene expression profiles of control and down-regulated PKCα expression in breast cancer cell lines by using siRNA technology. Classification of the down-regulated genes according to KEGG pathways rendered a big group related to *pathways in cancer*. Other important groups identified were *MAPK, ErbB and p53 signalling pathways*. Classification of up-regulated genes rendered *inositol phosphate metabolism* and *phosphatidylinositol signalling system* as the most important groups. These results suggest that PKCα is directly involved in the development of cancer by controlling important regulatory pathways. In addition, down-regulating PKCα promotes the increase of many other signalling proteins, which interact with phosphoinositols, suggesting that in these pathways PKCα acts as a break to control them.

P06-30
Synthesis of some 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine derivatives as possible protein kinase inhibitors

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Protein Kinases (PK), as being responsible for the phosphorylation of various proteins, play one of the major roles in cell signaling networks. Through chemically adding phosphate groups to proteins, PKs manage many cellular processes such as cell growth, proliferation, division, differentiation and apoptosis. That is to say, mutations and dysregulation of the kinase activity rise as a major mechanism in some diseases. To date, amplification, translocation, missense mutation and deletion of certain PK genes have been associated with numerous cancers. The catalogued 518 PK genes constitute a remarkable target for the

molecular cancer therapy. Therefore, the exploit of putative protein kinase inhibitors as drug candidates can be promising for the treatment of cancer. In recent years, studies reporting anticancer activities of a number of condensed 1,2,4-triazole derivatives such as triazolopyridazine, triazolotriazine, triazolothiadiazine via inhibition of various kinases, have being attracting interest. In this study, in the light of these knowledge, synthesis and screening possible anticancer activities of 1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazine derivatives is aimed. The required starting compounds, 4-amino-1,2,4-triazole-5-thiones, were obtained by the reaction of an acetic acid derivative with thiocarbohidrazide. The condensation of 4-amino-1,2,4-triazole-5-thiones with appropriate phenacyl bromides (or chlorides) in anhydrous ethanol under reflux gave 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines. In order to test cytotoxic activities of the compounds, sulforhodamine B (SRB) colorimetric assay was used. SRB assay provides correlative data about protein synthesis rate, hence cell proliferation. More than thirty small compounds were tested and three of them were found with cytotoxic activities within μM concentrations against HCT116 (colon), T47D (breast), Huh7 (HCC) cell lines. We then concentrated our study on HCC cell line panel with these three bio-active molecules. Their cytotoxicities were tested on Focus, HepG2, Hep3B, Huh7, Mahlavu, PLC, SkHep1, SNU-182 and SNU-475 cells. IC_{50} values were ~12–50 μM for FAT-TM, ~0.2–1 μM for CIAT-TM and ~7–23 μM for nAT-TM. The detailed SRB-based cytotoxicity results, shown as a time-dependent manner, indicated that these novel small molecules had strong anti-proliferative effects with promising anti-tumor activity on the HCC cell line panel we used. In the kinase assays we compared compounds to aminopurvalanol-A and staurosporine, which are well-known protein kinase inhibitors (PKI). The relative light units (rlu) obtained in both cases of well-known PKIs and our compounds are similar. Therefore, it could be concluded that these small-molecules possesses remarkable protein kinase inhibition potential. Further studies are envisaged to elucidate the detailed mode of action for the anticancer activity of these three small molecules.

P06-31
The localization of Sam68 and IRS1 interaction

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Sam68 (Src associated in mitosis) is a RNA binding protein that links cellular signaling to RNA processing. In previous studies we found that Sam68 interacts with IRS-1 in basal conditions without insulin stimulation. In this work we wanted to know where this interaction take place.

Both, BRET and co-immunoprecipitation assays after cell fractionation have been used for the study of Sam68 binding to IRS1. Inmunocytochemistry was used to corroborate BRET results.

BRET experiments indicated, that Sam68 associates with IRS1 manly in cytosol and in a lesser extension in nucleus.

By confocal microscopy we confirmed the two localizations for Sam68/IRS-1 interaction making the BRET results an easy way to quantify this interaction in order to relate it with possible patophysiological processes.

In conclusion, our data indicate that Sam68 interacts with IRS-1 in basal conditions in cytosol and nuclear localizations.

P06r-32
Azathioprine produces senescence through desensitization to IGF 1 in human hepatoblastoma cells

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Background and Aims: In children, the most common primary liver cancer is hepatoblastoma. The growth of this tumor is sensitive to insulin-like growth factor 1 (IGF-1). Most of the tumors evade cellular senescent. Our study focuses on the blocking of IGF-1 signaling on hepatoblastoma cells by azathioprine (AZA) and its subsequent effects. Methods: The cell lines HepG2 and Chang Liver were used. IGF-1 signaling was characterized by immunoprecipitation, by western blot and cell cycle analysis measured by flow cytometry. Senescent cells were measured by Senescence-associate (SA)-β-galactosidase (Gal) staining assay and by western blot of cell cycle proteins. Cellular necrosis was evaluated by LDH released assay. Apoptosis were measured by annexin V/propidium iodide staining and cleavage of PARP. Results: AZA treatment produced IRS-1 phosphorylation on Ser307 and IGF-1R dephosphorylation (even in presence of IGF-1) leading to their proteosomal degradation. Moreover, we observed the uncoupling between IRS-1 and p85 PI3K, which lead to the blocking of the IGF-1 signaling. As consequence, treatment with AZA produced the inhibition of AKT, p70S6K and FoxO1 even in presence of IGF-1. As previously described, dephosphorylation of the tumor supressor, FoxO1, inhibits cell cycle in G2/M and cellular proliferation. In addition, AZA increased the levels of p16, p21, Rb and c-myc, inducing cellular senescence.On the other hand, when HepG2 cells were co-treated with AZA and Bafilomycin A1(an inhibitor of H^+-ATPase from lysosome) senescence was inhibited and cells died by apoptosis. Conclusion: It has been shown that IGF-1 is a very important growth factor for the survival of tumors. Several drugs to treat cancer are being tested in clinical trials targeting the IGF-1 signaling. Our results suggest that AZA could be useful for the treatment of pediatric liver tumors in combination with other molecules, as Bafilomycin A1, because AZA inhibits IGF1-mediated signaling and sensitizes the cells to death.

P06-33
The relation between NGF and activin A is important for triggering pancreatic β cell apoptosis

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Activins, members of the transforming growth factor-β family, are pleitropic growth and differentiation factors. Activin A is known as inducer of β cell apoptosis. However, its relationship with other growth factors that have important role in the survival and functions of β cells like nerve growth factor (NGF) has not

been known yet. The aim of this study was to investigate the effects of NGF-withdrawal on synthesis and secretion of activin A from primary β cells and the importance of this relation for triggering β cell apoptosis which accepted as the key event of hyperglycemia and diabetes. In this study, β cells were isolated from euglycemic and streptozotocin-induced hyperglycemic rats and treated with NGF neutralization antibody for withdrawal of NGF in culture medium. Activin A levels in cell lysates and secretion samples were measured by western blotting and its gene expression level was determined by real time reverse transcription polymerase chain reaction assay. Moreover, Activin A expressed β cells were shown by double immunofluorescence technique. Apoptosis was quantitatively determined by cytoplasmic histone-associated DNA fragments. As the results, NGF neutralization caused β cells apoptosis; decrease in activin A at gene expression and protein levels; increase its secretion from β cells. Although the alterations seemed to be similar in euglycemic and hyperglycemic conditions, NGF withdrawal more strictly affected β cells of hyperglycemic rats. In conclusion, these important findings indicate that NGF is an important regulator for the synthesis and secretion of activin A from the β cells. Moreover, results suggested that NGF-withdrawal causes apoptosis by increasing activin A secretion from β cells of hyperglycemic rats.

P06-34
Regulation of fibulin-3 expression by C3G/p38alpha MAPK cascade in normal and tumour cells

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The subfamily of p38 MAPKs are activated by several stimuli leading to the regulation of different cellular functions, including migration and invasion. p38α MAPK is the most abundant isoform, being ubiquitously expressed and essential for embryonic development.

C3G is a guanine nucleotide exchange factor (GEF) for Rap1 and R-Ras proteins, but it has also functions independent of its GEF activity. It is essential for embryonic development due to its role in integrin-mediated adhesion and migration.

We have recently identified a functional interaction between C3G and p38α MAPK in the regulation of cell death in mouse embryonic fibroblasts (MEFs) and in chronic myeloid leukemia cells. In these systems, C3G acts through the inhibition of p38α MAPK in order to either activate or inhibit apoptosis, depending on the stimulus. In addition, we have found that this C3G/p38α cascade also regulates migration and/or invasion and the expression of some extracellular matrix proteins from the family of fibulins. In particular, fibulin-3, which can mediate invasion in certain tumour cells, is negatively regulated by p38α in MEFs (non-tumour cells) and the HCT116 colon carcinoma cell line. In contrast, C3G acts as a positive regulator of fibulin-3 expression in p38α Knock-out MEFs, while in HCT116 cells down-regulates fibulin-3 regardless of the presence or absence of p38α. Moreover, Rap-1 appears to be also a negative regulator of fibulin-3, but it does so through a C3G independent mechanism.

P06-35
Phosphorylated ATM at Ser-1981 (pATM) undergoes COPI mediated Golgi export upon double stranded DNA damage.

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The protein mutated in Ataxia-telangiectasia termed Ataxia-telangiectasia mutated (ATM) belongs to the family of phosphatidylinositol-3 kinase that is activated via autophosphorylation at serine 1981 following double stranded DNA damage. Activated ATM orchestrates signalling cascades that initiate DNA damage response (DDR) and govern cellular machinery that determines cell fate. Cells lacking ATM are hypersensitive to genotoxic stress induced through radiation or radiomimetic drugs. Here we report a novel DNA damage induced ATM trafficking mechanism that forms an integral component of DDR pathway. We have shown that in normal human keratinocytes, pATM is localised in Golgi and undergoes DNA damage induced Golgi export, contributing to its concomitant nuclear accumulation. This trafficking mechanism was found to be conserved in multiple cancer cell lines and was abrogated following inhibition of ATM kinase activity, demonstrating its kinase dependent autoregulatory role in trafficking during DDR. Furthermore, such inhibition also caused disruption of its co-localisation with β-COPI, coatomer protein involved in Golgi export. Since β-COPI recognizes its substrates via a di-Lysine recognition motif, we identified and mutated an N-terminal di-Lysine motif ^{387}-KK-^{388} within ATM protein to di-alanine. This mutation resulted in disruption of co-localisation between ATM and β-COPI and caused permanent Golgi retention and failure to undergo DNA damage induced nuclear accumulation of pATM. Hence, we have discovered a new aspect of ATM regulation, which involves ATM kinase dependent Golgi export of pATM mediated by β-COP1 during DDR. The elucidation of this trafficking event following double stranded DNA damage represents an additional mechanism of ATM regulation and provides another dimension through which DDR could be manipulated to achieve cellular sensitivity to genotoxic agents.

P06-36
Effect of IGF-I deficiency in the regulation of MEFs cellular functions

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Insulin-like growth factor I (IGF-I) is essential for neurogenesis and neuronal differentiation during inner ear development. IGF-I deficiency is associated with deafness in man and mice. In the cochlea of the *Igf1*-/- mouse, our group found out a decrease in AKt, Raf/ERK signalling and an increase in P-p38 levels. Furthermore, a high level of FOXM1 expression and a consequent decrease in p27 levels was also observed. p38 MAPKs are activated by several stimuli leading to the regulation of different cellular functions. p38α MAPK is the most abundant isoform, ubiquitously expressed and essential for embryonic development, playing a relevant role in stress responses. To further explore the mechanisms involved in *Igf1*-/- cochlear alterations and whether p38α plays any role, we generated primary mouse embryonic

fibroblasts (MEFs) and MEFs cell lines derived from *Igf1*+/+, *Igf1*+/- and *Igf1*-/- E13.5–14.5 mice. In addition, wt and p38α -/- MEFs were used for our studies. We found no significant differences in basal or IGF-I/insulin-induced proliferation in IGF-I-deficient MEFs, although there is a tendency of *Igf1* -/- MEFs to respond to IGF-I more efficiently. Moreover, activation of Akt, ERKs or p38 MAPK by IGF-I or insulin was similar in wt and *Igf1* -/- MEFs. Our data also suggest that the basal level of autophagy could be enhanced in *Igf1* -/- MEFs. We are currently characterizing this potential difference in autophagy. On the other hand, our data indicate that p38α would not be mediating the changes in FoxM1 and p27 observed in *Igf1* -/- cochlea. However, we are using other experimental approaches to deeply characterize the molecular mechanisms involved in IGF-I effects and its potential connection with p38α.

P06-37
Betacellulin is increased in absence of Nerve Growth Factor

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Betacellulin, a member of the epidermal growth factor family, expresses in pancreas and it increases beta cell mass. Nerve growth factor (NGF) regulates insülin secretion and plays an important role for pancreatic beta cell physiology. This study was aimed to investigate relationship between betacellulin and NGF. In this study, we used isolated beta cells from rat pancreas. There were two groups. First group was control group, and the other group was experimental group given streptozotocin. Each group divided into two subgroups as given NGF neutralization antibody or not. We showed betacellulin protein levels by western blotting in cell lysate and secretion, betacellulin mRNA levels by real time-PCR and cell proliferation by BrdU ELISA technique. As the results, betacellulin protein levels were significantly increased, while betacellulin mRNA levels were decreased in hyperglicemia group that non given NGF antibody (H-NGF) compared to control group that non given NGF antibody (C-NGF) in beta cell lysate. In secretion, betacellulin decreased at 0 hour in H-NGF compared to C-NGF, there was no alteration at 24 hour in H-NGF compared to C-NGF and decreased at 24 hour in hyperglicemia group that given NGF antibody (H + NGF) compared to control group that given NGF antibody (C + NGF). Beta cell proliferation was significantly decreased H-NGF and H + NGF compared to respectively C-NGF and C + NGF. In conclusion, NGF is an important growth factor for beta cell. In hyperglicemia, cell proliferation in the absence of NGF reduces more than in the presence of NGF. NGF also is important to release of betacellulin. Although betacellulin was decreased in both cell lysate and secretion in absence of NGF in hyperglicemia, betacellulin mRNA levels were increased. We thought that betacellulin may be increased to prevent damage that occurs by the absence of NGF.

P06-38
Cellular study of multiple kinase inhibitors in mutant EGFR driven NSCLC cell lines

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Mutant EGFR (epidermal growth factor receptor) driven pathways are playing important role in NSCLC (non-small cell lung cancer). Mainly patients harbournig somatic activating mutations (e.g. L858R) in EGFR respond efficiently to currently applied EGFR TKIs (tyrosine kinase inhibitors). However, in most cases patients relaps, due to secondary resistence mutations (e.g. T790M) in EGFR, or amplification of the oncogene MET (hepatocyte growth factor receptor gene).

Screening the molecular library of Vichem Ltd. in a recombinant kinase assay, we identified a TKI compound family (N-[4-(quinolin-4-yloxy)-phenyl]-sulfonamide derivatives) inhibiting activating mutant EGFR and c-Met kinases at various rates. Our scientific aim was to examine the anti-tumor potency and mechanism of action of these selected inhibitors, and the newly synthetised analogues. Therefore we utilized clinically relevant NSCLC cell lines (A549, H1975, HCC827, H1993) expressing c-Met and/or wild type or mutant EGFRs.

The IC50 values of the sulfonamide's in recombinant kinase assay were higher, than the reference compounds' IC50 values. However our molecules suppressed the proliferation of the NSCLC cell lines used, decreased their clonigenic ability and induced apoptosis according to flow cytometry experiments. Western blot analyses showed, that the sulfonamides indeed inhibit the phosphorilation of EGFR and c-Met kinases in the cancer cells, almost as potently, as the reference compounds.

We are going to characterise the compound's drug-likeness with ADME studies (PAMPA and CaCo-2 assays).

P06-39
The role of sphingosine 1-phosphate in the development of diabetic nephropathy

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Aim: To determine the role of sphingosine 1-phosphate (S1P) in the development of diabetic nephropathy.
Background: Hyperglycaemia and all TGFβ isoforms are involved in the development of diabetic nephropathy. S1P is derived from sphingosine by sphingosine kinase (SPHK-1/2) and is considered a key mediator of biological processes mimicking TGFβ signalling. However the role of SPHK1 in inducing fibrotic and inflammatory responses in diabetic nephropathy is unknown.
Methods: Human proximal tubular cells (HK2) were exposed to 5 mM, 30 mM D-glucose or 0.5 ng/ml TGFβ 1,2,3 in the presence or absence of a SPHK inhibitor (SKI-II; 2 μM) or SPHK1/2 siRNA (30 nM) for 72 hours. Exposure to S1P (0.1–5 μM) demonstrated the biological effect of SPHK activation. Wild type (WT) and SPHK1$^{-/-}$ mice with or without 24 weeks of diabetes were studied. In both *in-vitro* and *in-vivo* models, mRNA and protein expression of fibrotic and inflammatory markers were determined. Sphingolipid pathway metabolites were measured by mass spectrometry (MS).
Results: HK2 cells exposed to high glucose or TGFβ1,-2,-3 independently increased SPHK1 enzymatic activity, mRNA and protein expression. TGFβ1,-2,-3 and S1P induced fibronectin, collagen IV, chemokine ligand 2, vimentin and downstream phospho-p44/42 expression, which were reversed by both SKI-II and SPHK1/2 siRNA. WT diabetic mice exhibited a lower creatinine clearance, higher urinary albumin excretion, increased renal cortical fibronectin, collagen IV and phospho-p44/42 mRNA and protein expression compared to SPHK1$^{-/-}$ diabetic mice. MS demonstrated an increased in S1P in the kidneys of WT diabetic mice.

Conclusions: This study suggests limiting the formation of S1P inhibits renal inflammation and fibrosis in diabetic mice. This may be due to the reduced S1P receptor activation or indirectly due to inhibition of the p42/44 MAP kinase pathway.

P06r-40
Cell signaling and regulation by shedding of Death Receptor 6 (DR6)

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The apoptotic receptor DR6 belongs to the TNF receptor superfamily. Within this family, it is characterized by presenting a particularly long and glycosylated protein sequence (the stalk region) between its extracellular cystein rich domains (CRDs) and its transmembrane domain. Moreover, DR6 presents a Death Domain (DD) unusually close to the transmembrane domain as compared to other Death Receptors. Towards the C-terminal region DR6 contains a long cytoplasmic tail without known protein domains. The functional study of this receptor has revealed its implication in apoptotic processes especially in nervous system cells, although it also appears to play an important role in the immune system. DR6 triggers signaling pathways leading to the activation of JNK or NF-κB. An important aspect of DR6 regulation which remains essentially unknown is the rapid shedding of the receptor in which its extracellular region is released from the plasma membrane to the extracellular medium. It has been suggested that DR6 shedding is involved in dendritic cells modulation. Also, the extracellular region of DR6 has been suggested to act as a tumor biomarker. In order to study the ability of DR6 to activate JNK/AP-1 pathway in HEK293 (non transformed) and HeLa (transformed) cells we have generated DR6 mutant receptors with different deletions in its intracellular domain. Using this approach we can demonstrate the important role that the DR6 cytoplasmic tail plays in this signaling pathway, being the most C-terminal 75 aminoacids of the receptor required to activate JNK/AP-1. On the other hand, we generated another set of mutant receptors with different deletions in its extracellular stalk region which affect the various glycosylation sites of this region. These mutant receptors have allowed us to study DR6 shedding. As expected, shedding occurs very close to the transmembrane domain although there are indications that more than one cleavage site may exist. Furthermore, it seems that the glycosylated extracellular region of DR6 could play a regulatory role in the shedding process.

P06-41
From autoinhibition to inhibition in trans: structure-function studies on the novel mechanism of Raf-1:Rock2 kinase cross-talk

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Conditional gene ablation studies with epidermis restricted Raf-1 ablation in mice have proven the essential role of Raf-1 in the maintenance of Ras-driven epidermal tumours. Raf-1 can exert this essential function via pathway cross-talk, directly interacting with Rock2 kinase and inhibiting its activity. In their inactive states, the activity of each kinase is kept in check via inhibiton by its own regulatory domains. After autoinhibiton is relieved, the regulatory domain of Raf-1 can bind to and inhibit the activity of Rock2 kinase domain in trans. In the cell, both kinases can form homo/heterodimers. Analysis of the formation of Raf-Rock complex using dimerisation-dead mutants of the kinases showed no direct role of dimerisation in Raf-Rock interaction. To determine the functional' (inhibitory) domain orientation in the Raf-Rock complex, we used drug-induced dimerization to force the interaction between the Rok kinase and Raf regulatory domain in different orientations. Of the orientations investigated, the one where the cysteine-rich domain (CRD) of Raf formed interaction with the active site of the Rok kinase domain inhibited Rock2 most effectively, fully consistent with our proposed model for the interaction in trans. The detailed characterisation of the Raf-Rock interaction will pave the way for designing small molecule inhibitors against the interaction using as a (co-)therapy for Ras-driven epidermal tumours.

P06-42
Exploring GRK2 involvement in DNA Damage Response and its pathophysiological consequences

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DNA damage response (DDR) is a complicated network of DNA repair mechanisms, damage tolerance processes, and cell-cycle checkpoint pathways aimed to deal with genomic lesions that can arise in normal cellular processes (DNA replication, metabolic expenditure) or after exposure to environmental insults (radiation, alkylating reagents). Accurate and efficient regulation of DDR is critical for genomic integrity and a potent anti-tumorigenic barrier. In fact, defects in the ability to properly respond to and repair DNA damage underlie many forms of cancer. The DDR pathway involves the orchestration of DNA-damage sensors (MRN, BRCT complexes), initiator/mediators (ATM/ATR, Chk1/2) and effectors (p53), which are regulated by complex phosphorylation-based cascades. We have recently found that GRK2 (G-protein coupled Receptor Kinase 2) is part of an intrinsic pathway that ensures timely progression of cell cycle at G2/M. Moreover, responsiveness of p53 to DNA damage during G2 progression was modulated by the expression level and functionality of GRK2. Despite this kinase has been classically involved in GPCR desensitisation and internalisation, emerging evidence now supports that GRK2 is able to initiate alternative signaling pathways that might influence proliferation and the efficacy of cell cycle checkpoints in different pathological contexts. Accordingly, increased levels of GRK2 have been found in diverse neoplastic diseases as human granulose cell tumors, thyroid and prostate cancer or some breast tumors. We provide evidences that GRK2 is involved in the regulation of DNA damage response (DDR), by negatively modulating the activation of the ATM downstream effectors Chk2 and p53 in response to doxorubicin exposure. Interestingly, GRK2 protein levels correlate inversely with the ability of the cell to enter a senescent phenotype. Our results unveil a novel role of GRK2 in DDR pathway regulation that might have important repercussions in malignant transformation and tumour response to chemotherapeutic treatments.

P06r-43
Involvement of the yeast Ppz1 protein phosphatase and potassium homeostasis in flocculation and invasive growth

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The yeast *Saccharomyces cerevisiae HAL3* and *VHS3* genes encode two multifunctional proteins, able to work both as inhibitory subunits of the Ppz1 Ser/Thr phosphatase and in the coenzyme A biosynthetic pathway. The *hal3* and *vhs3* deletions are synthetically lethal due to their role in CoA biosynthesis. A phenotypic characterization of a conditional *tetO:HAL3 vhs3* mutant revealed that, under semi-permissive conditions, this strain displays a flocculent phenotype dependent on the Flo8-mediated increased expression of the flocculin Flo11, as well as invasive growth. We show here that deletion of *PPZ1* in the conditional mutant eliminates these phenotypes. Moreover, we have found that Ppz1-dependent flocculation and invasive growth is mediated by the PKA pathway, specifically by the the Tpk2 catalytic subunit isoform. The observation that the *tetO:HAL3 vhs3* strain presents a higher concentration of cAMP would explain the activation of the PKA pathway and would constitute the link between *FLO11* expression and Ppz1 activation. Our results indicate that a strain lacking Trk1/2, the high-affinity potassium transporters displays a moderate invasive phenotype. Because hyperactivation of Ppz1 results in inhibition of potassium uptake, our data supports a functional link between potassium homeostasis and the invasive phenotype. We are currently developing a possible model that connects potassium homeostasis with the regulation of the activation state of the PKA pathway. Work supported by grants EUI2009-04147 (SysMo2) and BFU2011-30197-C3-01 to J.A.

P06-44
The phosphorylation of PLD1 on Thr147 increases PLD1 activity resulting in Der f 2 - induced IL-13 production via ROCK1 binding to ATF-2 in Beas-2B cells

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The major house dust mite allergen, Der *f* 2 is well known causative agent of atopy or allergic conditions, which involved many inflammatory cytokines expression. Previously, we reported that PLD1 (phospholipase D1) plays an important role in Der *f* 2 - induced IL-13 production in human bronchial epithelial cells (Beas-2B). In the present study, we focused on how PLD1 activation is regulated by p38 MAPK, and then a mechanism between PLD1 and activating transcription factor-2 (ATF-2) in Der *f* 2- induced IL-13 production. Treatment of Der *f* 2 activated the p38 MAPK, and dominant negative or p38 MAPK inhibitor decreased Der *f* 2- induced IL-13 expression and production. Der *f* 2- induced PLD1 activation was attenuated by p38 MAPK inhibition. This result indicates that p38MAPK act as upstream activator of PLD1 in Der *f* 2- treated Beas-2B. Interestingly, expression and activation of IL-13 increased by Der *f* 2 were decreased by the PLD1 T147V mutant, and a constitutively active mutant (PLD1 T147E) promotes Der *f* 2- induced IL-13 expression and production, indicating that phosphorylation of PLD1 on Thr147 by p38MAPK is important for IL-13 expression and production. The introduction of dominant negative RhoA or RhoA siRNA or treatment with Y-27632, a Rho kinase-specific inhibitor, attenuated IL-13 expression and production in response to Der*f* 2treatment. By immunoprecipitation, ATF-2 was physically associated with ROCK1. Binding of the ROCK1 to ATF-2 was enhanced in Der*f* 2-stimulated cells, indicating that ATF-2 activation links to the ROCK1. Taken together, our findings demonstrate that the phosphorylation of PLD1 on Thr147 by p38 MAPK up-regulates PLD1 activity which consequently activates ATF-2 through binding with the ROCK1resulting in IL-13 expression and production in Der*f* 2-treated BEAS-2B cells.

P06r-45
Hxk2 nuclear-cytoplasmic shuttling

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Hexokinase 2 (Hxk2) from *Saccharomyces cerevisiae* was one of the first metabolic enzymes described as a multifunctional protein. Hxk2 has a double subcellular localization and role, it functions as a glycolytic enzyme in the cytoplasm and as a regulator of gene transcription of several Mig1-regulated genes in the nucleus. However, the mechanism by which Hxk2 enters in the nucleus was unknown until now. Here we report that the Hxk2 protein is an import substrate of the carriers α-importin (Kap60 in yeast) and β-importin (Kap95 in yeast). We also show that the Hxk2 nuclear import and the binding of Hxk2 with Kap60 are glucose-dependent and involve one lysine-rich NLS, located between lysine-6 and lysine-12. Moreover, Kap95 facilitates the recognition of the Hxk2 NLS1 motif by Kap60 and both importins are essential for Hxk2 nuclear import. It is also demonstrated that Hxk2 nuclear import and its binding to Kap95 and Kap60 depends on the Gsp1-GTP/GDP protein levels. Thus, our study uncovers Hxk2 as a new cargo for the α/ β-importin pathway of *S. cerevisiae*. In the same way, we try to determinate the general rules of Hxk2 nuclear-cytoplasmic transport. We have identified Ser14 as a relevant residue for the transport regulation. The interaction between Hxk2 and the transport proteins Kap60 and XpoI, and consequently, it movements through the nuclear membrane, depends on the phosphorylation state of Ser14. This phosphorylation is glucose-dependent, been the absence of the hexose the signal for phosphorylation. Thus, we assure that Ser14 is a key regulator of the Hxk2 nuclear-cytoplasmic shuttle. Moreover, we identify the regulatory enzymes implicated in the Ser14 phosphorylation.

P06-46
Glycolytic inhibitors (Ionidamine, 2-deoxy-D-glucose) as chemo-sensitizing agents in leukaemia cell models. Differential regulation by oxidative stress, and LKB-1/AMPK, Akt/mTOR and MEK/ERK signalling pathways

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Glycolytic inhibitors represent valuable sensitizing agents for cancer therapies, often with cell type- and drug-dependent effects.

We here demonstrate that clinically useful concentrations of lonidamine (Lon, 50–100 μM) and 2-deoxy-D-glucose (2-DG, 2–10 mM) potentiate apoptosis induction by the arsenic trioxide (ATO, Trisenox) and other anti-tumour drugs in leukaemia cells, although with different pattern of specificity. Using ATO and HL60 as drug and cells models we observed that: (i) Lon and 2-DG rapidly cause inner mitochondrial membrane permeabilization, which nevertheless does not correlate with apoptosis generation. On the other hand apoptosis generation by Lon/ATO and 2-DG/ATO correlates well the activation of factors regulating the mitochondrial executioner pathway. (ii) Lon (but not 2-DG) stimulates reactive oxygen species production, which explain in part ATO sensitization. (iii) 2-DG (but not Lon) causes ATP depletion, which nevertheless does not adequately explain anti-tumour drug sensitization. (v) Lon causes late activation of defensive MEK/ERK and Akt/mTOR pathways, while 2-DG rapidly causes IGF-1R-mediated activation these pathways. The activation is in both cases prevented by ATO. (vi) Lon stimulates LKB-1/AMPK, while 2-DG inhibits this pathway. This inhibition, which favours apoptosis, is mediated by Akt and ERK activation. In summary, Lon and 2-DG operate as efficacious sensitizing agents in leukaemia cells, although with non-coincident regulatory mechanisms. MEK/ERK and Akt/mTOR activation reduce the efficacy of Lon and 2-DG as chemotherapeutic drugs, and are therefore targets of pharmacologic intervention. Inactivation of these pathways by ATO in part explains the efficacy of the combined treatments, suggesting a possible clinical application.

P06-47
A receptor guanylyl cyclase mediating Toll-independent/dMyD88-dependent humoral response and dMyD88-independent cellular response in Drosophila immunity

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The fruit fly *Drosophila melanogaster* is an excellent model organism to decipher the basic principles of innate immune responses consisting with humoral and cellular responses. One of the humoral responses is induction of antimicrobial peptides (AMPs), that is controlled by two distinct innate immune signaling pathways, the Toll and immune deficiency (imd) pathways. The Toll pathway is mediated by Toll receptor and dMyD88 adaptor protein, the imd pathway is mediated by Imd adaptor protein. The cellular responses are mainly caused by blood cells called hemocytes. To identity genes capable of activating immune responses, we have established a genome wide gain-of-function genetic screen in *Drosophila* and identified peptidoglycan recognition protein -LE recognizing diaminopimeric acid-type peptidoglycans and activating imd-dependent AMP synthesis and autophagy (PNAS 2002, EMBO J. 2004, Nature Immunol. 2006, 2008). Here, using the genetic screen, we identified a receptor guanylyl cyclase (rGC). The rGC shows amino acid sequence similarity to mammalian receptor guanylyl cyclases producing cyclic GMP such as Atrial Natriuretic Peptide receptor. The rGC is involved in both Toll-independent/dMyD88-dependent humoral response and dMyD88-independent cellular response, that is crucial for host survival against Gram-positive bacterial infections but not against Gram-negative bacterial infections.

P06-48
PKC activity regulates motility and is involved in the maintenance of plasma membrane organization in boar spermatozoa

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Protein kinase C (PKC) is a key regulatory enzyme in signal transduction mechanisms. Diacylglycerol and phorbol esters, which stimulate PKC activity, enhance the onset of the early stages of the acrosome reaction in mouse, human, ram and bovine spermatozoa. PKC activator phorbol-12-myristate-13-acetate (PMA) stimulates boar sperm protein Ser phosphorylation in specific PKC recognized motifs while it does not affect acrosome reaction. Our aim is to study the effect of PMA on boar sperm motility and plasma membrane function under non-capacitating (TBM) or capacitating (TCM) conditions. Spermatozoa viability, plasma membrane scrambling (PMS) and the acrosome functional status were analyzed by flow cytometry. Motility was analyzed by the ISAS® program. PMA treatment increases PMS from 13.6 ± 1.2% to 24.5 ± 3.1% in TBM and from 28.2 ± 4.4% to 43.1 ± 5.4% in TCM, this effect was lower compared with a cAMP analogue 8Br-cAMP. This PMA effect in PMS is blocked by H89, a protein kinase A inhibitor, indicating a crosstalk between PKA and PKC pathways. Moreover, PMA induces head-to-head spermatozoa agglutination with intense flagellar beating. PMA-induced PKC activity decreases the percentage of rapid spermatozoa (average path velocity > 80 μm/s) from 64 to 47% (TBM) and from 61% to 35% (TCM). Our data show that PKC activity in boar spermatozoa reduces the percentage of rapid spermatozoa and increases plasma membrane scrambling, which could be responsible for the head-to-head agglutination Supported by Grants: MICINN AGL2010-15188 and GR10156-JUEX

P06r-49
Using genetic approaches to gain insight into the functions of the Ptc1 type 2C phosphatase in the yeast *Saccharomyces cerevisiae*

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Type 2C Ser/Thr phosphatases (PP2Cs) are a conserved group of monomeric enzymes that in S. cerevisiae are encoded by seven related genes (PTC1–7). Cells lacking Ptc1 display multiple phenotypes compared with other mutants of the PP2C family suggesting that Ptc1 play specific roles in many regulatory pathways that are not shared by other PP2Cs. However, besides its well-known function in the osmotic responsive pathway as Hog1 phosphatase, other cellular targets of Ptc1 remain still unknown. To gain further insight into the functional role(s) of Ptc1, and assuming that the phenotypes ascribed to the lack of Ptc1 could be the result of hyperphosphorylation of specific substrates, we have combined the ptc1 mutation with the entire collection of viable kinase mutants and we have screened for rescue of nine different ptc1 phenotypes. A matrix of phenotype intensities has been created and analyzed. Interestingly, the most general and intense genetic interaction has been found for the mkk1 mutation, thus confirming previously established links between Ptc1

and the cell wall integrity (CWI) pathway. The results of a parallel screen for high-copy gene suppressors of the rapamycin-sensitive phenotype of cells lacking Ptc1 further supports a functional interaction between Ptc1, TOR and the CWI signaling pathways. These genetic approaches might be instrumental in shedding light on the identification of specific target(s) for Ptc1.

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P06-50
Fenofibrate enhances the barrier function of endothelial continuum within the metastatic niche of prostate cancer cells

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Efficiency of cell extravasation, which is an initial step of cancer metastasis, depends on the invasive potential of circulating cancer cells, but also on the local functional status of endothelial continuum in the proximity of cancer cells. Using an *in vitro* experimental model of such a 'metastatic niche' of prostate cancer cells based on the co-cultures of DU145 cells with human umbilical vein endothelial cells (HUVEC), we demonstrated the local impairment of the barrier function of endothelial continuum in the proximity of cancer cells paralleled by the activation of HUVEC motility, cytoskeleton rearrangements and recruitment of vinculin to focal adhesions. Fenofibrate stabilized endothelial continuum in the proximity of prostate cancer cells (PCC) and attenuated its penetration by cancer cells. This effect was correlated with an inhibition of HUVEC motility, paralleled by the development of mature, vinculin-rich focal adhesions and the upregulation of vinculin expression. The inhibition of PPAR activity abrogated the effect of fenofibrate on HUVEC cytoskeleton architecture but not on cell motility. Since HUVEC motility was restored by N-acetyl-L-cysteine, these observations indicate the involvement of PPAR-independent mechanisms, determined by reactive oxygen species, in the regulation of HUVEC motility by fenofibrate. Thus, we suggest that the signaling pathways which determine cytoskeleton architecture, motility and barrier function of endothelial cells within metastatic niche are affected by fenofibrate. Our data provide a mechanistic rationale for extending the clinical use of the well tolerated PPAR agonists and for its combination with existing multidrug regimens used in prostate cancer therapy. This work was supported by funds granted to the Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University to further The Development of Young Scientists and Doctoral Students 2011/2012 (PSP:K/DSC/000365, DS/46/2011) and by the Polish National Science Centre (grants 2011/01/B/NZ3/00004).

P06-51
Hcm1, a forkhead transcription factor involved in mitochondria metabolism, acts as a nutrient sensing factor and is regulated by phosphorylation in yeast

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The forkhead transcription factor family in *Saccharomyces cerevisiae* consists in four members, Fkh1, Fkh2, Hcm1 and Fhl1. These factors have been mainly related to the regulation of the cell cycle. Hcm1 is expressed periodically during cell cycle with a peak of expression in G1/S. This factor regulates chromosome segregation genes and fills the S-phase gap in the transcriptional circuitry of the cell cycle. In mammals, members of the Forkhead box O (FOXO) class of transcription factors are key players in the regulation of cell-fate decisions, such as cell death, cell proliferation and cell metabolism. These factors are strictly regulated by posttranslational modifications, like phosphorylation, ubiquitination and deacetylation. In our group we have progressed in the study of Hcm1. We showed that this factor is not only involved in cell cycle, but it's also related to oxidative stress, respiratory metabolism and mitochondria biogenesis. In fact, the overexpression of Hcm1 is enough to shift cells from a fermentative metabolism to respiration. Our latest work demonstrates that Hcm1 acts as a nutrient sensing factor, because its cellular localization (from cytosol to the nucleus) and protein level, respond to glucose and nitrogen. We have also studied the regulation of Hcm1 by several kinases involved in metabolism. In this way, we analyzed Hcm1 cellular localization in Δ*snf1*, Δ*tor1*, and Δyak1 strains. Moreover, we performed *in vitro* phosphorylation assays to demonstrate that Hcm1 is directly phosphorylated by these kinases. Taken together, our results indicate that Hcm1 is not only a cell cycle regulator, as it is involved in other processes. These biological processes are linked to each other and how this is regulated remains to be elucidated.

P06-52
Effect GHRH and JMR-132, on proliferation, migration and adhesion in an experimental model *in vitro* of human prostate cancer

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Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males. The expression of GHRH has been demonstrated in several cancers suggesting that GHRH may be involved in the pathogenesis and growth of tumors. JMR-132, a more recent and highly potent GHRH antagonist, has been shown to inhibit cell growth in some human carcinomas. In this regard, the effectiveness of several GHRH antagonists on tumor growth reduction has been demonstrated. In this work, we analyze the effect of GHRH and JMR-132, in human androgen-independent prostate cancer PC3 cells. First, we performed a dose-response curve by MTT to choose the optimal treatment concentration of GHRH and JMR-132. Then, we performed BrDU incorporation, adhesion with collagen IV and wound healing cell assays after treatment with GHRH (10^{-7} M)

and JMR-132 (10^{-7} M). We observed an increase of viability, proliferation and migration, and a decrease of adhesion in GHRH treatment cells. However, the treatment with JMR-132 showed a decrease on viability, proliferation and migration, and a increase on adhesion assays. These results support the relevance of GHRH as a potential therapeutic target, as well as the effectiveness of GHRH antagonist, JMR-132, as inhibitor of the progression tumoral in experimental human prostate cancer. This work was supported by grants from Comunidad Autónoma de Madrid/Universidad de Alcalá (CCG08-UAH/BIO-3782) and Junta de Castilla-La Mancha (PII10-0189-3222).

P06-53
Ceramide-1-phosphate stimulates carbohydrate metabolism

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Ceramide-1-phosphate (C1P) is a bioactive sphingolipid that is implicated in the regulation of cell growth and survival, as well as in the control of cell migration. All of these actions require high energy levels to be available in the cells. We hypothesized that C1P might stimulate glucose uptake and metabolism so that cells could generate sufficient ATP to accomplish these vital biological functions. Here we show that C1P stimulates the incorporation of glucose by macrophages and that it enhances the production of ATP. C1P-stimulated glucose transport involved the glucose transporter GLUT3 and was insulin-independent. Investigation into the mechanism by which C1P exerts this action revealed that the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt) is a major pathway involved in this process. In addition, we observed that C1P-stimuated glucose uptake and metabolism were inhibited by pertussis toxin, a potent Gi protein inhibitor that is commonly used to block cell signaling through activation of Gi protein-coupled receptors. Taken together, these results demonstrate that C1P-stimulated glucose uptake is mediated by GLUT3 and is dependent upon activation of the PI3K/PKB pathway. This action may also involve the interaction of C1P with a putative plasma membrane receptor that is coupled to Gi proteins in macrophage plasma membranes.

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P06-54
The ISA virus increases ROS levels via the MAPK pathway

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Infectious salmon anemia (ISA) is a disease that primarily affects Atlantic salmon (*Salmo salar*) in captivity. The virus of infectious salmon anemia (ISAv) is the etiologic agent responsible for the disease that causes severe economic losses in the salmon industry. ISAv is a single strand and segmented negative sense RNA virus of the family Orthomyxoviridae viruses like influenza. This virus has a putative virulence protein HE (hemagglutinin) associated with HRP (Highly Polymorphic Region).The sequencing of the isolated Chilean has shown ISAv HRP7 and HRP2 highly virulent. In this regard, the virus has the ability to infect, replicate and cause cytopathic effect (CPE) in different cell lines of salmon, in fact it is documented that ISAv induces apoptosis as the final step in the mechanism of infection and interestingly viral infection of the family Orthomyxoviridae is associated with redox changes characteristic of oxidative stress. Previous results from our laboratory show that ISAv increased reactive oxygen species (ROS) in cells of salmon through the NADPH oxidase complex in short periods of incubation and this cell phenomenon is associated with decreased enzyme stabilizing cellular oxidative stress. We evaluated the MAPK proteins as signal transduction pathway, a possible connector between the virus and the production of ROS. Through the infection of primary culture of salmon and total head kidney cells with an isolate of ISAV national, ROS production and testing of real-time qPCR, we assessed the involvement of MAPK proteins in the course of the ISA virus infection. Our observations suggest that the ISA virus increases the production of ROS and additionally there is an increased transcript level of MAPK protein. These results demonstrate for the first time that ISAV retains the ability to produce oxidative stress in cells infected fish, similar to viruses affecting mammals.

P06-55
Involvement of the nuclear proteasome activator PA28γ in the cellular response to DNA double-strand breaks

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The DNA damage response (DDR) is a complex signaling network that leads to repair of the damage while modulating numerous cellular processes. DNA double-strand breaks (DSBs) – a highly cytotoxic DNA lesion – activate this system most vigorously. The DSB response network is orchestrated by the ATM protein kinase, which phosphorylates key players in its various branches. Proteasome-mediated protein degradation plays an important role in the proteome dynamics following DNA damage induction. Here, we identify the nuclear proteasome activator PA28γ (REGγ; PSME3) as a novel DDR player. PA28γ depletion leads to cellular radiomimetic sensitivity and alters dynamics of damage-induced nuclear foci of phosphorylated histone H2AX (γH2AX) or foci formed by damage response proteins such as MDC1, 53BP1and BRCA1. Specifically, PA28γ-deficiency abrogates the balance between the two major DSB repair pathways – nonhomologous end-joining and homologous recombination repair. Furthermore, a fraction of PA28γ is recruited to the sites of DNA damage and is required for rapid accumulation of proteasomes at these sites. Moreover, PA28γ is found to be an ATM target and its phosphorylation kinetics correlate with the kinetics of its accumulation on the chromatin fraction. Our data reveal a novel ATM-PA28γ-proteasome axis of the DDR that is required for timely coordination of DSB repair.

P06-56
Phosphorylation of Ser 203 of RCAN3 increases its binding affinity to CnA

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The RCAN family of proteins are endogenous regulators of the activity of calcineurin (Cn) on the NFAT family of transcription factors that among others, mediates the activation of the immune response. In humans, there are three RCAN: RCAN1, RCAN2 and RCAN3. Two motifs have been described to be implied in Cn binding and regulation: the LXXP and the CIC motifs. Within the CIC motif the PXIXIT sequence competes with the PXIXIT motif of NFAT for the same binding site on Cn without affecting Cn general phosphatase activity. The CIC motif is the direct responsible of Cn binding and inhibition of NFAT-dependent cytokine gene expression and, as a consequence, T cell activation. Thus, CIC-derived peptides have immunosuppressant potential. As we have previously observed that the full length RCAN proteins are *in vivo* phosphorylated by protein kinase CK2 at one serine residue conserved at the CIC motif of RCANs, we wondered whether this phosphorylation could affect the affinity to Cn. We early reported a biologically active RCAN3 CIC peptide ($RCAN3^{183–203}$) and here we explored a derivative that includes the CK2 phosphorylation site (S/TxxE/D – peptide $RCAN3^{183–208}$). We used this peptide to analyze the influence of the phosphorylation state of Ser 203 on the peptide affinity to CnA, the catalytic subunit of Cn. Fluorescence polarization assays showed that phosphorylated Ser 203 in $RCAN3^{183–208}$ peptide has an increased affinity to CnA when compared to the unphosphorylated peptide and a better profile as competitive peptide for the interaction between CnA and the NFATc2 SPRIEIT peptide (PXIXIT motif). These results were further confirmed using increasing amounts of the phosphorylated or unphosphorylated $RCAN3^{183–208}$ peptides to compete the interaction of GST-NFATc2 with endogenous Cn by pull down analysis. Elucidating the molecular mechanisms that regulate RCAN proteins and its functional effect on the Cn-NFAT signaling pathway could be important in the development of novel therapeutic tools for the immunosuppressive therapy. Supported by SAF009-08216, BFU2009-10189 and 2009SGR1490 grants. A.A. has a FI fellowship, S.M-H from IDIBELL and S.S-S. a FPU fellowship.

P06-57
Studying the interaction between lysophosphatidic acid and the PH domain of gelsolin

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In signaling processes many multicomponent complexes are formed under the cell membranes, in which numerous proteins and lipids co-exist. The modular structures of these proteins allow the formation of protein-protein and lipid-protein interactions in signaling complexes. Nowadays, many domains are known as parts of signaling proteins among eukaryotes. One of them is Pleckstrin Homology (PH) domain, which binds phosphoinositide derivatives hereby regulating the functions of PH domain containing proteins. Formerly it was described that gelsolin, an actin filament severing protein, can be regulated by binding phosphoinositide-(4,5)-bisphosphate (PIP_2) to its PH domain and lysophosphatidic acid (LPA) can interfere with PIP_2 for the lipid-binding site. It is well-known that in eukaryotic cells LPA, a physiologically important mediator of cell survival and migration, acts on specific cell surface receptors (edg-2, edg-4, edg-7) as a first messenger. However, it can also activate the nuclear receptor PPARg as well as affect membrane processes via mostly unidentified target proteins and mechanisms. Our aim is to characterize the binding properties of gelsolin to LPA through its PH domain. *In vitro* fluorimetric studies suggest that gelsolin (and its PH domain) can interact with LPA only when the lipid is in bundled form. The apparent K_d is in the micromolar range, a strong effect could be detected about the critical micelle concentration of LPA (25–50 μM). A much weaker interaction was detected in case of other structurally similar bioactive sphingolipids, which shows the selectivity of lipid binding. Reverse titration experiments indicated, however, that the real binding constant of the LPA-PH domain interaction is in the low nanomolar range. These data are correlated well to our previous binding studies with calmodulin and sphingosylphosphorylcolin as well as β-2-microglobulin and LPA, raising the possibility of a novel lipid-protein interaction type in which the lipid partner is in an associated form.

P06-58
Transcription of liver X receptor is down-regulated by 15-deoxy-δ12,14-prostaglandin J2 through oxidative stress in human neutrophils

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Liver X receptors (LXRs) are ligand-activated transcription factors of the nuclear receptor superfamily. They play important roles in controlling cholesterol homeostasis and as regulators of inflammatory gene expression and innate immunity, by blunting the induction of classical pro-inflammatory genes. However, opposite data have also been reported on the consequences of LXR activation by oxysterols, resulting in the specific production of potent pro-inflammatory cytokines and reactive oxygen species (ROS). The effect of the inflammatory state on the expression of LXRs has not been studied in human cells, and constitutes the main aim of the present work. Our data show that when human neutrophils are triggered with synthetic ligands, the synthesis of LXRa mRNA became activated together with transcription of the LXR target genes ABCA1, ABCG1 and SREBP1c. An inflammatory mediator, 15-deoxy-Δ12,14-prostaglandin J2 (15dPGJ2), hindered T0901317-promoted induction of LXRa mRNA expression together with transcription of its target genes in both neutrophils and human macrophages. This down-regulatory effect was dependent on the release of reactive oxygen species elicited by 15dPGJ2, since it was enhanced by pro-oxidant treatment and reversed by antioxidants, and was also mediated by ERK1/2 activation. Present data also support that the 15dPGJ2-induced serine phosphorylation of the LXRa molecule

is mediated by ERK1/2. These results allow to postulate that down-regulation of LXR cellular levels by pro-inflammatory stimuli might be involved in the development of different vascular diseases, such as atherosclerosis.

P06-59
PTPIP51 interacts with NFκB p65 subunit – the balance between life and death?

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Protein Tyrosine Phosphatase Interacting Protein 51 (PTPIP51) is an evolutionary conserved protein, which regulates proliferation, differentiation, mitosis and apoptosis. PTPIP51 exerts this multiple cellular functions by interaction with distinct protein under the influence of protein kinases and protein tyrosine phosphatases. So, PTPIP51 regulates the mitogen activated protein kinase (MAPK) pathway by the interaction with Raf-1 through members of the 14-3-3 protein family under the control of protein tyrosine phosphatase 1B. Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NFκB) is a specific transcription factor, which is critical in the regulation of immune response, cell regulation and apoptosis. It is a central part of the TNF-alpha induced signaling cascade, which is subject to the therapy of various autoimmune diseases as Crohn's disease or rheumathoid athritis. NFκB constists of five known subunits, one of them is named NFκB p65 subunit, or RelA. RelA resembles a transcription factor. This factor negatively regulates pro-apoptotic genes. Moreover, RelA is reported to interact with the extracellular signal regulated kinase 1 (Erk-1) linking the NFκB pathway to the MAPK pathway. PTPIP51 and NFkB are co-localized in HaCaT cells. By Duolink proximity ligation assay the interaction of both proteins was verified. Furthermore, the dynamic change of the PTIP51/RelA and RelA/Erk-1 interaction is investigated under the stimulation of TNF-alpha in HaCaT cells. In conculsion, this new established interaction possibly resembles a new link of both pathways – NFκB and MAPK pathway – to control cell growth and survival.

P06r-60
Ret signaling in Smith-Lemli-Opitz syndrome

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The tyrosine kinase receptor Ret is implicated in survival, migration and differentiation of neuronal populations from both central and peripheral nervous systems. It has also been described to be essential for the development of the genito-urinary system. In mice, loss of function mutations of Ret are associated with developmental defects that include ptosis, Hirschprung disease and kidney malformations among others. Lipid rafts are cholesterol-enriched membrane domains that function as signaling platforms in many cellular systems. Optimal bioactivity of several receptor tyrosine kinases including Ret relies on lipid raft integrity. Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive malformation syndrome characterized by an inborn error in the cholesterol synthesis. Among other defects, it has been shown that lipid rafts are disrupted in patients affected by SLOS. Interestingly, SLOS patients show congenital malformations that are consistent with impaired Ret signaling, such as ptosis, colonic aganglionosis and renal malformations. In this work we sought to analyze whether Ret signaling is altered and implicated in the pathogenesis in a mouse model of SLOS. We show evidence that Ret signaling is diminished in cultured sympathetic neurons and kindey explants from *Dhcr7*-/- mice.

P06r-61
Effect of STIM1 phosphorylation at ERK1/2 target sites on myoblast migration and myotube formation

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Calcium signaling is known to have a key role on myotube formation, and store-operated calcium entry (SOCE) has been proposed to be involved in the control of Ca^{2+} entry at the onset of molecular events required to trigger myogenesis. SOCE is tightly regulated by STIM1, a transmembrane endoplasmic reticulum protein that activates store-operated Ca^{2+} channels (SOCs) when intracellular stores are depleted in response to a variety of stimuli. We have previously shown that phosphorylation of STIM1 at Ser575, Ser608, and Ser621, which are ERK1/2 target sites, modulates SOCE. To study the role of STIM1 phosphorylation at these sites on myoblast migration and myotube formation we used C2C12 cells transiently transfected with tagged-STIM1, wild-type or mutated at the aforementioned residues, either to glutamate or alanine to mimic constitutive phosphorylation or dephosphorylation, respectively. We found that (i) STIM1 is phosphorylated at these sites in C2C12 myoblasts and that myoblasts expressing Flag-STIM1$^{S575A/S608A/S621A}$ migrate slower than cells overexpressing wild-type STIM1 in wound-healing assays. (ii) Conversely, glutamate substitution mutation (Flag-STIM1$^{S575E/S608E/S621E}$) strongly promotes migration, and this effect is inhibited by SKF96365, a well-known inhibitor of SOCs. (iii) In addition, we found that phosphorylation of Ser575 increases significantly, and that Ser621 becomes dephosphorylated during myotube formation. In sum, phosphorylation of STIM1 modulates both migration and myotube formation, although the kinetics and profiles of phosphorylation are different in both events.

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P06r-62
Role of BCRP in FXR-induced chemoresistance in liver and intestinal cancer cells

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The usefulness of pharmacological anticancer treatment is often reduced by up-regulation of genes involved in mechanisms of chemoresistance, including ABC export pumps. The aim of this study was to investigate the ability of the nuclear receptor FXR to activate these mechanisms in response to antitumor drugs. Liver (Alexander) and intestinal (LS174T) cancer cells were transfected with vectors containing the ORF of human FXR, RXR, BCRP and/or firefly luciferase. Gene expression was determined by RT-QPCR. Cell viability was measure by MTT assay. Expression of FXR in Alexander and LS174T cells was negligible. Transfection with human FXR/RXR induced a significant reduc-

tion in the sensitivity to cisplatin. This protective effect was further enhanced by the FXR agonist GW4064. In Alexander cells, transfection with FXR/RXR also protected against colchicine, doxorubicin, mitomycin C and potassium dichromate, but not against artesunate, paclitaxel and acetaminophen. In these cells, both GW4064 and cisplatin were able to induce the expression of FXR target genes, such as BSEP, SHP, and OSTβ. Moreover, in HepG2 cells, that constitutively express FXR, and in Alexander FXR/RXR transfected cells, cisplatin induced luciferase expression led by an IR-1 element. In primary cultures of human hepatocytes, cisplatin also induced the expression of FXR target genes. Transfection of LS174T with FXR and treatment with GW4064 induces BCRP up-regulation. In contrast, this does not occur in Alexander transfected cells, human hepatocytes and HepG2 cells treated with either cisplatin or even GW4064. Moreover, transfection of LS174T cells with the ORF of BCRP enhanced the resistance of these cells to cisplatin and mitoxantrone, a known substrate of this ABC protein. In conclusions, bile acid-independent activation of FXR can be triggered by exposure to some toxic compounds, which may result in enhanced chemoresistance of liver and colon cancer cells. In intestinal cells, but not in liver cells, the FXR-induced protective effect against cisplatin may be mediated in part by up-regulation of BCRP.

P06-63
Src kinases catalytic activity regulates proliferation, migration and invasiveness of MDA-MB-231 breast cancer cells

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SFKs are frequently deregulated in cancer where they control cellular proliferation, migration, survival and metastasis. Here we study the role of SFKs catalytic activity in triple-negative/basal-like and metastatic human breast cancer MDA-MB-231 cells employing three well-established inhibitors: Dasatinib, PP2 and SU6656. These compounds inhibited migration and invasion. Concomitantly, they reduced Fak, paxillin, p130CAS, caveolin-1 phosphorylation and altered cytoskeletal structures. They also inhibited cell proliferation, but in different manners. Dasatinib and PP2 increased p27(Kip1) expression and reduced c-Myc levels, restraining G1–S transition. In contrast, SU6656 did not modify p27(Kip1) expression, slightly altered c-Myc levels and generated polyploid multinucleated cells, indicating inhibition of cytokinesis. These later effects were also observed in SYF fibroblasts, suggesting a SFKs-independent action. ZM447439, an Aurora B kinase inhibitor, produced similar cell cycle and morphological alterations in MDA-MB-231 cells, indicating that SU6656 blocked Aurora B kinase. This was confirmed by inhibition of histone H3 phosphorylation, the canonical Aurora B kinase substrate. Furthermore, hierarchical clustering analysis of gene expression profiles showed that SU6656 defined a set of genes that differed from Dasatinib and PP2. Additionally, Gene Set Enrichment Analyses revealed that SU6656 significantly reduces the Src pathway. Together, these results show the importance of SFKs catalytic activity for MDA-MB-231 proliferation, migration and invasiveness. They also illustrate that SU6656 acts as dual SFKs and Aurora B kinase inhibitor, suggesting its possible use as a therapeutic agent in breast cancer.

P06-64
Surface vimentin involved in the cell entry of SARS-CoV

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During the period of 2002–2003, severe acute respiratory syndrome associated coronavirus (SARS-CoV) caused a global panic due to its high morbidity and mortality as well as worldwide incidence. Soon after, the deadly disease outbreak, the functional cellular receptor was determined as angiotensin-converting enzyme 2 (ACE2). However, ACE2 itself is not sufficient for viral entry, other host factors are likely to be involved in the infection process. In this study, a host intracellular filamentous cytoskeletal protein vimentin was identified to be involved in spike-ACE2 interaction by performing immunoprecipitation assay coupled with LC/MS/MS analysis. More notably, incubating cells with virus-like particles (VLPs) of SARS-CoV for a specific time frame, the surface vimentin level was increased. A direct interaction between spike and vimentin was further confirmed by Western blot analysis. In addition, antibody neutralization assay demonstrated the functional role of vimentin in cell uptake of SARS-CoV VLPs. These results indicate that vimentin directly interacts with SARS-CoV spike protein and associates with the spike-ACE2 complexes on the plasma membrane, which leads to the cell entry of SARS-CoV VLPs. This study demonstrates a clear association of vimentin with SARS-CoV infection, and indicates the possibility of the vimentin as a new target for vaccine development.

P06r-65
Dioxin receptor controls C-terminal Src kinase (CSK)-binding protein (CBP) signaling to caveolin 1 and β1 integrin to modulate cell adhesion and migration

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Recent studies strongly support a regulatory role for the dioxin receptor (AhR) in cell adhesion and migration. Following our previous work, we report here that the C-terminal Src kinase-binding protein (Cbp)-Csk-Src pathway sustains migration of transformed fibroblasts (T-FGM) and regulates ®1 integrin activation and caveolin-1 (Cav1) tyrosine phosphorylation, and that such mechanism is AhR dependent. T-FGM *AhR*-/- fibroblasts

had higher integrin ®1 activation that coincided with higher Cbp expression, increased fibronectin secretion and impaired directional migration. Notably, interfering Cbp/Pag1 expression in *AhR*-/- fibroblasts rescued ®1 integrin activation, migration and cell morphology. c-Src activation (Tyr^{416}) was inhibited, probably because the increase in Cbp/Pag1 levels produced an accumulation of inhibitory Csk- Cbp/Pag1 complexes. Consistently, focal adhesion kinase (FAK) phosphorylation was reduced at Tyr^{576}-Tyr^{577}. The c-Src target Cav1 was hypophosphorylated at Tyr^{14}, and its association to c-Src diminished in *AhR*-/- cells. Interestingly, AhR was present at the plasma membrane in detergent resistant microdomains (DRM; rafts) and co-immunoprecipitated with Cav1 under basal cell conditions, revealing a functional link by which AhR could alter Cav1 distribution between DRM and non-DRM domains. Furthermore, AhR interference impaired directional migration and Cav1 distribution. Thus, fibroblasts migration requires AhR for proper regulation of the Cbp/Pag1-dependent signaling to ®1 integrin and Cav1.

P06r-66
Galphaq interaction with the novel effector PKCz is essential for the activation of the ERK5 pathway and is negatively regulated by GRK2

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The cell is a dynamic entity with highly intertwined biochemical networks responding to internal and external perturbations in an orchestrated manner. The elements in those networks are usually proteins that build complex circuits through protein-protein interactions. G proteins are essential cellular components for the signalling events following G protein-coupled receptor (GPCR) activation. This function is achieved through specific and evolutionary conserved interactions between activated G proteins and a number of cellular effectors. Particularly, the Galphaq/11 family of G proteins has been classically shown to associate to and activate the enzyme PLCbeta. However, a growing body of evidence points at additional effectors to be responsible for some of the functions of Galphaq. We have recently reported that GPCR activation promotes the interaction between Galphaq and two novel effectors, PKCz and MEK5, thereby activating the ERK5 pathway. This is an important mechanism in the cardiovascular system and in the development of cardiac hypertrophy. Here we present a biochemical characterisation of the Galphaq/PKCz protein complex with specific focus on the interaction surfaces and the negative modulation by the GPCR kinase 2 (GRK2), a well-known player in Galphaq signalling. We identified lysine 19, located in the PB1 domain of PKCz, and two glutamic acids at positions 234 and 245 located in the switch III domain of Galphaq, to be essential for the formation of the Galphaq/PKCz complex. Introduction of the double mutation (E234A/E245A) in a constitutively active form of Galphaq completely abrogated ERK5 activation. Additionally, we found that GRK2, through its interaction with Galphaq, prevents the association to PKCz, impairing the downstream activation of ERK5 by Galphaq-coupled GPCR. Overall, this study provides important biochemical insight into the activation of ERK5 by Gq-coupled receptors and also puts forward GRK2 as a key regulator of the pathway.

P06-67
Involvement of a novel signaling molecule, PRIP in the regulation of reproduction system

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Phospholipase C-related but catalytically inactive protein (PRIP), composed of type 1 and type 2, was first identified as a novel D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] binding protein, but the biological functions have remained elusive. We therefore generated PRIP-1 and –2 double knockout (DKO) mice to gain insight into the biological function. DKO mice showed dysfunction of the reproductive system in the female such as decreased number of pups, longer estrous days, increased secretion of gonadotropins, etc. We examined the ability of the ovaries in response to gonadotropins: the total number of the ovulated oocytes were remarkably decreased in DKO female mice, indicating that PRIP plays an important role in follicle maturation and/or ovulation, but not fertilization or implantation. Microarray and quantitative real-time PCR analyses revealed the increased expressions of molecules involved in ovulation including Has2, Ptgs2, PTX3, Tnfaip6 and Ereg. We examined the ability of COC expansion in both genotypes but little difference was observed. On the other hand, histological analysis of ovaries from both genotypes revealed more immature follicles and fewer mature follicles or corpus lutea in DKO ovaries. Immunofluorescence analysis showed the expression of luteinizing hormone receptors (LHR) at earlier stages of follicle maturation in DKO ovary and more increase of antral follicles. These results are summarized as below: the increased LH secretion from pituitary gland and the appearance of LHR at immature follicle stages lead excessive LH signaling to suppress follicle maturation in DKO females, indicating that PRIP may be involved in positive regulation of ovarian follicle maturation through LH signaling.

P06-68
β-arrestin1, a key epigenetic regulator in pediatric leukemia stem cells?

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Leukemia is the most common childhood malignancy, and acute lymphoblastic leukemia (ALL) is the main subtype of pediatric acute leukemia. Though the long-term disease-free survival (DFS) and overall survival (OS) rate of pediatric leukemia has been improved, the relapse is often occurred. The personalized therapy that based on patients' risk stratification and prognosis is pivotal for reducing relapse. Some factors are important for childhood ALL risk stratification, and need investigation. Leukemia stem cells (LSCs) are critical for minimal residual disease monitoring, as well as independent prognostic factors. Based on our prior results of the tumorigenic role of β-arrestins, the multifunctional scaffold protein mediating many intracellular signaling pathways, we further investigated its role in leukemia, which is little known previously. Here, the abnormal expression and aberrant regulation of β-arrestin1 was found in pediatric leukemia, especially in LSCs, from several studies. The functional role of β-arrestin1 in regulating the self-renewal of LSCs through DNA methylation will be further presented and discussed. This could provide a novel leukemiagenic mechanism and potential new therapeutic target for ALL.

P06-69
Increasing extracellular pH stimulates PKB/Akt and MAPKs in perfused rat hearts

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Insulin is important in the regulation of cardiac protein mass and is one of the most powerful stimulators of cardiac protein synthesis rate (k_s). Insulin signals exclusively through phosphoinositide 3'-kinase (PI3K) and PKB/Akt in heart and activation of this pathway is responsible for increasing k_s. However, in perfused rat hearts, raising extracellular pH (pH_o) from pH 7.4 (Krebs-Henseleit buffer, Tris or HEPES) to pH 8.2 (Tris) stimulates k_s to the same extent as insulin. We hypothesised that pH_o 8.2 would stimulate the PI3K-PKB/Akt signaling pathway in perfused hearts. pH_o 8.2 stimulated PKB(T308) or PKB(S473) phosphorylation over 10 – 30 min, and was about half as effective as insulin. The effects of pH_o 8.2 were abolished by the PI3K inhibitor, LY294002. In addition, pH_o 8.2 stimulated phosphorylation of downstream effectors of PKB/Akt [p70S6K(T389), S6(S235/S236), S6(S240/S244), 4E-BP1(T37/T46), GSK3α(S21) and GSK3β(S9)] to about the same extent as insulin. We also examined whether pH_o 8.2 resulted in phosphorylation (activation) of MAPKs. Perfusion at pH_o 8.2 (30 min) resulted in phosphorylation of ERK1/2, JNKs and p38-MAPK (insulin was without effect). Whereas phosphorylation of ERK1/2 or p38-MAPK was maximal at 10 min and then declined, phosphorylation of JNKs was slower but was maintained over 60 min. ERK1/2 phosphorylation was inhibited by the MKK1/2 inhibitor, PD184352. pH_o 8.2 also caused phosphorylation of the JNK substrate, c-Jun(S63), and this was inhibited by the JNK inhibitor, SP600125. Similarly, pH_o 8.2 resulted in phosphorylation of the p38-MAPK substrate, MAPKAPK2(T222) and this was inhibited by the p38-MAPK inhibitor, SB203580. We hence conclude that pH_o 8.2 activates multiple signalling pathways in the hearts. We hypothesise that the insulin receptor-related receptor (InsRR), the third member of the insulin receptor family and which has recently been identified as a pH_o sensor in kidney, may play a role in some of the effects of pH_o 8.2. We therefore also showed that InsRR protein is expressed in the heart, and its abundance is about 50% of that in kidney.

P06-70
E-cadherin gene C-160A promoter polymorphism and risk of colorectal cancer in a Turkish population

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Colorectal cancer is the second most frequent cause of cancer-related death. The process of colorectal tumorigenesis is multistep genetic model. The human E-cadherin the major cadherin molecule expressed by epithelial cells.

A total of 100 paraffin-embedded colorectal cancer specimens were obtained from department of pathology in Cerrahpasa Medical Faculty. Also a total 100 paraffin-embedded normal tissue was used from same cases as a control group. We performed PCR-restriction fragment length polymorphism (RFLP) to analyze the polymorphism. Ten-micrometer-thick tissue sections were placed on a glass slide and stained with HE. Then the tissue sections were dehydrated in graded ethanol solutions and dried without a cover glass. DNA was extracted from the tissues with 100 μl of extraction buffer at 55°C overnight. The tubes were boiled for 10 min to inactivate the proteinase K. After amplification with the same primer set, the PCR products were digested with restriction enzyme *Hinc*II. All analyses were performed using the Statistical Package for the Social Sciences (SPSS).

In conclusion, we observed a statistically significant relationship between the evaluation of E-cadherin alterations by PCR-RFLP and stage of tumors. The combined effects of gene-to-gene and gene-to-environmental risks significantly increase the risk of developing colorectal cancer. Therefore, further studies are needed to confirm our results.

P06-71
SHP1-dependent signaling platforms formation regulates prostatic cells migration

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Prostate cancer is a frequent malignancy among men in industrialized countries, occupying the first place of cancer incidence in the European Union. Tumours begin as an uncontrolled cellular proliferation, followed by local invasion and dissemination to other tissues, process known as metastasis. During these steps cells should be able to recognize and migrate through different kinds of extracellular matrix being a key event for tumour progression. SHP-1 is a tyrosine phosphatase present in haematopoietic tissues, being also expressed in epithelial cells where it controls cell cycle progression. Our objective was to analyze the role of SHP-1 on cell migration and the molecular mechanisms underlying this effect. We silenced its expression in several cell lines derived from prostatic metastases and we assessed their migratory capacity through collagen or fibronectin. In PC3 cells, SHP-1 depletion caused a lower number of migrating cells with regard to control. In these cells, SHP-1 coinmunoprecipitated with molecules implicated in cell migration such as FAK, p85 subunit of PI3K and AKT1. Furthermore, SHP-1 depletion decreased FAK phosphorylation at Tyr397 and abrogated PI3K activity and consequently, AKT1 phosphorylation at Ser473 and Thr308. Surprisingly, SHP1 depletion in LNCaP cells promotes cellular migration through fibronectin and although SHP-1 coinmunoprecipitated with FAK, SHP-1 knockdown did not affect the Y397 FAK phosphorylation. In these conditions, AKT1 phosphorylation on S473 and T308 was decreased. In conclusion, SHP-1 regulates the migration of prostate cancer cell by interaction with molecules implicated in this cellular process, modifying its activity.

P06-72
TGR5 in macrophages is protective against atherosclerosis and insulin resistance

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Bile acids (BAs) are amphipathic molecules essential to emulsify dietary lipids and fat-soluble vitamins and prepare them for subsequent absorption. In addition to their established role in dietary lipid absorption, BAs have also emerged as important signaling molecules that control various aspects of metabolic homeostasis. Both nuclear and membrane receptors mediate the transduction of bile acid signals into adaptive cellular responses. While the

genomic effects of BAs are mainly governed by the nuclear receptor FXR, many of the rapid non-genomic effects have been attributed to activation of the membrane receptor TGR5.

TGR5 is highly expressed in macrophages, and activation of TGR5 inhibits LPS-induced inflammatory cytokine secretion through a TGR5-cAMP-NF-kB pathway. Using $LDLR^{-/-}TGR5^{-/-}$ double knockout mice, we demonstrated that TGR5 activation inhibits atherosclerosis. The reduction in atherosclerotic lesions were associated with less lesion macrophage content and decreased intraplaque inflammation. We also observed that TGR5 inhibits oxidized LDL loading in macrophages, which is in line with the reduced expression of the scavenger receptors CD36 and SR-A. Transplantation of $TGR5^{-/-}$ bone marrow into $LDLR^{-/-}$ recipients confirmed that the protective effect of TGR5 activation on atherosclerosis was mediated through TGR5 in leukocytes. Macrophages also have a central role in the pathogenesis of insulin resistance and obesity. We observed that $TGR5^{-/-}$ mice on high fat diet become obese and insulin resistant, and display increased inflammatory cytokine levels. Together, these data suggest that TGR5 in macrophages is an attractive target against various low-grade inflammatory disorders, including atherosclerosis and insulin resistance.

P06-73
Interaction of lysophosphatidic acid with PH domains

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In signaling the activation of cell-surface receptors leads to selective gene transcriptions or immediate physiological reactions. In these processes many multicomponent complexes are formed at the membranes containing several proteins as well as membrane-lipids. The structure of signaling proteins is modular by which domain-domain or domain-lipid interactions are evolved. Some of these domains are responsible for lipid binding. The aim of this study is to understand the mechanistic details of some protein – lipid interactions in signaling. Several membrane-associating proteins contain domains which directly interact with lipids, in our case plecktsrin homology (PH) domains. Our attention turned to lysophospholipid mediators, especially lysophosphatidic acid (LPA), as it previously has been reported that LPA binds to the PH domain of gelsolin, possibly interfering the inositol polyphosphate binding of this domain. In the course of our work we aimed at characterizing the interaction between several PH domains and LPA using biochemical and biophysical methods *in vitro*. After expression of the PH domain and the entire protein of gelsolin, fluorescence and CD spectroscopy as well as isothermal titration calorimetry measurements indicated that LPA can bind to the PH domain of gelsolin over its critical micelle concentration. This interaction is specific to LPA. Based on our findings we have looked for other PH domain containing proteins, namely Vav2, Akt1 and Grp1, seeking for similar interactions. We expressed the PH domains of the proteins and characterized their interaction with LPA. We also carried out live-cell confocal microscopy with GFP-tagged PH domains of Akt1 and Grp1. We studied the changes in spatiotemporal localization of these GFP-PH domains by modifying the intracellular level of LPA. Our results show that LPA can act a second messenger-like fashion on membrane surfaces via recruiting protein domains. LPA, in a clustered form mimicked by micelles *in vitro*, can bind to at least some PH domains, revealing new aspects of lysophospholipid-protein interactions.

P06-74
Crosstalk between Ras and LKB1 in glycine *N*-methyltransferase-deficient hepatocellular carcinoma

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Background & Aims: Hepatocellular carcinoma (HCC) is the leading cause of death in cirrhotic patients. Liver cirrhosis at a high risk of HCC has abnormal S-adenosylmethionine (SAMe) levels. Glycine N-methyltransferase (GNMT), responsible for catabolism of SAMe, is often silenced in HCC. Mice lacking GNMT develop fibrosis and HCC, exhibit epigenetic changes of critical carcinogenic pathways that support an essential role of GNMT in HCC.
Methods: Here we have studied the role of LKB1-AMPK and RAS in the proliferation and transformation of GNMT-deficient HCC.
Results: GNMT-deficient HCC was characterized by LKB1-AMPK misconnection, leading to resistance to apoptosis mediated by a positive regulation of cAMP-PKA-CaMKKβ cascade. Additionally, Ras-mediated hyperactivation of LKB1 contributed to the proliferation of GNMT-deficient HCC in an ERK/p90RSK-dependent manner. The observed LKB1-induced Ras activation was due to the regulation of RASGRP3 expression. Notably, human HCC tumors with poorer prognosis showed the lowest levels of *GNMT*, p-AMPKα(Thr172) and the highest activation of Ras/LKB1/RASGRP3 axis.
Conclusions: The present data shows a correlation between LKB1 and RAS activity in a context of HCC with low *GNMT* expression, indicating that activation of the RAS/LKB1/RASGRP3 cascade might possess an important prognostic role in human liver cancer. Furthermore, these findings open the possibility to design new therapeutic strategies for the treatment of liver cancer.

P06-75
mAChR modulate adhesion and migration in human breast cancer cells

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Previous studies have shown that stimulation of muscarinic acetylcholine receptors (mAChR) induces an increase in the growth and proliferation of MCF-7 human breast cancer cells by activa-

tion of the mitogen activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) cascade. In this study, we investigated the role of mAChR in the regulation of cell-substratum adhesion and migration of MCF-7 cells. We found that short-term stimulation of mAChR did not increase alpha- and beta-integrins expressed at the surface of MCF-7 cells, but augmented the cell adhesion to extracellular matrix (ECM) protein collagen type I, fibronectin, vitronectin and laminin, which was prevented by pretreatment of cells with atropine, a mAChR antagonist. Cell-substratum adhesion was also blocked by PP2 and LY294002, but no by pretreating of cells with PD98059 or GF109203X. These data suggest that Src-family tyrosine kinases and phosphatidylinositol 3-kinase (PI3K), but not MAPK/ERK and protein kinase C (PKC), could participate as mAChR intracellular intermediary in the specific activation of integrin receptors. In addition, in MCF-7 human breast cancer cells mAChR stimulation induced an increase of matrix metalloproteinase 9 (MMP-9) activity, but not of MMP-2 activity and tissue inhibitor of metalloproteinases 1 and 2 (TIMP-1/2) activity, and also produced a reduction of serum-stimulated cell migration. Taken together, our data indicate that mAChR modulates the adhesion and migration of MCF-7 cells, which could play a crucial role in the malignant progression of human breast cancer cells.

P06-76
LPS activates motility of macrophages that requires participation of Src tyrosine kinases

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LPS is a component of the outer membrane of Gram-negative bacteria which strongly activates production of pro-inflammatory mediators by macrophages. We found that LPS at 100 ng/ml rapidly induces also polarization, ruffling and direct movement of J774 cells; all these phenomena were seen already after 20–30 min of cell stimulation. The two receptors of LPS, CD14 and TLR4, translocated to the leading lamella of polarized cells, where they transiently colocalized. Concomitantly, polymerization of actin filaments took place at the cell front. The leading edge was also heavily decorated with a PLC-PH-GST probe indicating local accumulation of $PI(4,5)P_2$, a lipid controlling activity of several actin-binding proteins. LPS stimulated phosphorylation of two actin regulatory proteins, paxillin on tyrosine 118 and N-WASP on serine 484/485. Phosphorylation of these proteins was rapid peaking after 20 min of stimulation of cells with 100 ng/ml LPS. Both phospho-proteins concentrated at the leading edge of the cells. LPS-induced protein phosphorylation was inhibited by PP2, a drug affecting activity of kinases of the Src family. However, activity of Lyn, one of major Src kinases in macrophages, indicated by phosphorylation of tyrosine 396 of the catalytic domain of Lyn and dephosphorylation of inhibitory tyrosine 507, peaked only after 30–60 min of stimulation of cells with 100 ng/ml LPS. Taken together the data suggest that activity of some of kinases of the Src family is required for LPS-induced reorganization of the actin cytoskeleton with possible participation of Lyn kinase in later stages of these events.

P06-77
Identification of DUSP10 as a Cyclooxygenase 2 activity induced gene and its role in colon cancer progression

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Colorectal cancer is one of the deadliest types of cancer, due to its high metastatic capacity and chemoresistance acquisition. Cyclooxygenase 2 (Cox-2) elevated expression has been shown to play an important role in colorectal tumour development and its inhibition can help prevent cancer death. We sought to identify mechanisms that mediate the pro-tumorigenic effects of Cox-2 generating cell lines that stably overexpress this gene and performed gene expression microarray analysis to identify genes up- or down-regulated by this overexpression. We identified genes with altered expression in these conditions, one of them being the dual specificity phosphatase DUSP10, specific for JNK and p38 MAPKs. DUSP10 resulted to be up-regulated by Cox-2 activity as well as by PGE2 treatment in a concentration depended manner. We generated cell lines stably expressing DUSP10 by infecting with lentivirus the colorectal adenocarcinoma HT29 cells. We found that DUSP10 overexpression stimulated cell proliferation and colony forming capacity. Moreover, DUSP10 overexpression increased cell stress resistance, a characteristic shared with Cox-2 overexpression.When DUSP10 overexpressing cells where xenografted subcutaneously in nude mice, the resulting tumours grew faster in size. These results suggest that DUSP10 expression can confer a growth advantage to colorectal cancer cells.

P06r-78
Function and regulation of GRK2 in tumor progression of stratified epithelia

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Introduction: G-protein-coupled receptor kinase 2 (GRK2) causes desensitization of GPCRs, such as chemokine receptors, turning down their signaling. It has been described that reduced GRK2 levels potentiate leukocyte chemotaxis, and emerging evidence suggest that GRK2 expression modulates epithelial cell migration in a cell-type and stimulus-dependent manner. Our main focus has been to study the functional and regulation of GRK2 expression in tumors of stratified epithelia. Results and Discussion: Interestingly, we detect GRK2 expression in differentiated areas of these tumors but not in the invasive front areas. Moreover, there is a significant negative correlation between GRK2 expression and tumor malignancy and grade, that could be useful in tumor prognosis. In this regard, overexpression of GRK2 in spindle-like cells induces an epithelial phenotype, and preliminary results indicated that reduced GRK2 levels increase migration of human keratynocytes. Moreover, the skin of GRK2 hemizygous mice displays an altered pattern of expression of genes involved in cell cycle and cell proliferation. We also find that in the skin of these animals there is a down-regulation of miR-145, a tumor supresor miRNA that directly targets several proteins involved in cancer and inhibits proliferation. Conclusion: our results support a putative inhibitory role of GRK2 in tumoral progression in stratified epithelium.

P06-79
Identification of PMEPA1 as a cyclooxygenase 2 induced gene and its potential implication in cancer progression

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Cyclooxygenase 2 (Cox-2) elevated expression has been associated with tumour development especially in colorectal cancer and it is well established that Cox-2 inhibition can help prevent cancer. In order to study the mechanisms that mediate the protumorigenic effects of Cox-2 we generated cell lines stably overexpressing this gene and performed gene expression microarray analysis to identify genes up- or down-regulated by this overexpression. PMEPA1 is one of the up-regulated genes in the Cox-2 overexpressing cells. PMEPA1 was originally identified as a highly androgen-induced gene with abundant expression in several cancers. The encoded product is a transforming growth factor-β (TGF-β)-induced transmembrane protein overexpressed in breast, prostate and colorectal cancer. We found that PMEPA1 expression can be induced by PGE2, the principal prostaglandin produced in the Cox-2 overexpressing cells, as well as by TGF-b. In view of these observations, we overexpressed PMEPA1 in human colon adenocarcinoma (HT29) and human ovarian carcinoma (SKOV3) cells, generating stable cell lines. We studied *in vivo* and *in vitro* cell characteristics, noting significant differences between empty vector (EV) and PMEPA1 overexpressing cells. The actin cytoskeleton distribution was markedly different in PMEPA1 overexpressing Skov3 cells resulting in differences in cell morphology. *In vitro* proliferation and colony formation assays showed that PMEPA1 overexpression reduced cell proliferation rates. Mouse xenograft experiments, with subcutaneous injection of the mentioned cells in nude mice, showed a differential effect of PMEPA1 overexpression in the two cell types, HT29 and SKOV3, leading to a final growth delay of HT-29 xenografts, while PMEPA1 overexpressing SKOV3 cells had a high initial survival and proliferative advantage compared with EV cells. These findings suggest that PMEPA1 may have an important role in cancer progression.

P06r-80
Phosphorylation of RCAN proteins by protein kinase CK2 enhances their inhibitory role towards the calcineurin-NFATc signaling pathway

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Cyclosporine A and FK506 achieve immunosuppression by blocking calcineurin- dependent NFATc activation. However, their long-term application is associated to severe side effects. A novel human RCAN-derived peptide, known as CIC sequence, has been shown to inhibit NFATc activation without affecting general phosphatase activity of calcineurin (Cn). Therefore, it is postulated that this peptide and its derivatives have a highly specific immunosuppressant activity. CK2 has been described to regulate Cn-NFAT signalling by regulating NFAT phosphorylation in the nucleus. Here, by *in vitro* and *in vivo* radiolabeling techniques, we show that CK2 phosphorylates a conserved serine residue within the functional CIC-motif of vertebrate RCANs, and that this phosphorylation increases its inhibitory potential on NFATc-dependent promoter activity and on NFATc- dependent gene expression in activated human T cells. Our findings led us to postulate a novel regulatory mechanism by which protein kinase CK2 regulates Cn-NFATc pathway via a phosphorylation in a conserved serine of human RCAN proteins, rendering a more potent NFATc-signalling inhibitor. The knowledge of the modulation of the Cn-NFATc pathway provided by the RCANs may help in the development of novel immunosuppressant drugs, more selective and potent than the currently used in immunosuppressive therapy.

P06-81
Differential signalling through p90 ribosomal S6 kinases (RSKs) to cardiomyocyte gene expression by endothelin-1 vs alpha-adrenergic agonists

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Endothelin-1 (ET-1) or α-adrenergic receptor agonists (e.g. A61603) promote cardiomyocyte hypertrophy. Both signal through Gq protein-coupled receptors and extracellular signal-regulated kinases1/2 (ERKs) to induce changes in gene expression. However, although there is overlap, the transcriptomic responses are not identical. ERKs phosphorylate transcription factors (TFs) and activate p90 ribosomal S6 kinases (RSKs) that phosphorylate (different) TFs. Inhibition of RSKs with BID1870 inhibits ~55% of RNAs upregulated by ET-1 (1 hour) in cardiomyocytes. Thus, a major part of the ERK signal to gene expression is via RSKs. We investigated activation profiles and subcellular localisation of ERKs/RSKs in cardiomyocytes to determine if there are differences in degree of activation or isoform selectivity in the response to ET-1 versus A61603. ET-1 increased phosphorylated (activated) ERKs in cytosolic (C) and nuclear protein-enriched (NPE) fractions within 30 sec (maximal: 2–3 min) with no difference in activation profiles. ERK phosphorylation induced by A61603 was less than that induced by ET-1 and was delayed. The NPE fraction contained ~25% of total ERKs, but there was no net change in distribution following stimulation with either agonist. ET-1 promoted phosphorylation of RSKs within 1 min. The NPE fraction contained ~8% of total RSKs in control cells but, on stimulation, this increased to 34% with ET-1 or 18% with A61603, indicating that activation is associated with nuclear translocation. ET-1 increased RSK1 and RSK2 isoform content in the NPE fraction whereas A61603 only increased RSK2 content. The greater degree of activation of ERKs/RSKs by ET-1 may account for a greater overall transcriptomic response, but differential activation of RSKs may lead to different profiles.

P06-82
Critical role of murine protein serine/threonine kinase 38 (MPK38)-mediated phosphorylation of serine-threonine kinase receptor-associated protein (STRAP) at Ser188 in STRAP-mediated cell death

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Murine protein serine/threonine kinase 38 (MPK38) coimmunoprecipitates with serine-threonine kinase receptor-associated protein (STRAP), a transforming growth factor-β (TGF-β) receptor interacting protein, and this association is mediated by the carboxyl-terminal regulatory domain of MPK38 and the fourth to sixth WD40 repeats of STRAP. Using cysteine-to-serine amino acid substitution mutants of MPK38 and STRAP, we demonstrate that $Cys^{339}$and $Cys^{377}$of MPK38 and $Cys^{152}$and $Cys^{270}$of STRAP are required for MPK38-STRAP binding. The association between MPK38 and STRAP was significantly increased by TGF-β or MPK38/ASK1 signals (H_2O_2, TNF-α, thapsigargin, and ionomycin). MPK38 phosphorylates STRAP at Ser^{188}, suggesting a potential role for STRAP phosphorylation in the MPK38-mediated regulation of STRAP activity. MPK38 alleviates STRAP-mediated inhibition of TGF-β and ASK1 signaling in a kinase-dependent manner. MPK38 also positively and negatively regulated STRAP-induced p53 and PDK1 signaling through STRAP phosphorylation at Ser^{188}, respectively. These results indicate that MPK38 functions as a regulator of STRAP activity and function through direct interaction with and phosphorylation of STRAP, leading to stimulation of apoptosis.

P06-83
NESI regulates the subcellular localizations of nuclear-cytoplasmic transport proteins CAS and importin-alpha

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Nuclear export signal-interacting protein (NESI) plays a critical role in the nuclear export of large hepatitis delta antigen that possesses a proline-rich NES. NESI contains seven potential transmembrane domains and mainly localizes at the perinuclear region. However, biological roles of the NESI protein involved in the nuclear-cytoplasmic transport of cellular proteins are not known. In this study, CAS that is known to function as a shuttle protein to recycle importin-alpha from the nucleus to the cytoplasm was identified to be associated with NESI following coimmunoprecipitation and LC/MS/MS analysis. To understand whether NESI is involved in the nuclear-cytoplasmic transport of CAS and importin-alpha, shRNA specific to NESI was introduced to hepatocellular carcinoma Huh7 cells. Immunostaining demonstrated an accumulation of CAS and importin-alpha in the nuclei of *nesi* knockdown cells, whereas they were localized to the cytoplasm in the absence of NESI shRNA. Hydrogen peroxide induced a distribution of importin-alpha to the nucleus and overexpression of NESI led a partial localization of importin-alpha to the cytosol. Furthermore, importin-alpha mediates translocation of NF*k*B into the nucleus. NF*k*B localized to both the cytoplasm and the nucleus in Huh7 cells. NF*k*B was mainly distributed to the cytoplasm in *nesi* knockdown cells, whereas it translocated to the nucleus when NESI was overexpressed. These results indicate that NESI plays regulatory roles in the nuclear-cytoplasmic transport of cellular proteins under various physiological conditions.

P06-84
A new in-gel phosphatase assay using fluorogenic substrates and its application

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A variety of biological processes in animals, plants, and microorganisms are regulated by protein phosphorylation. Intracellular signaling networks are known to be constructed on the basis of the subtle balance between phosphorylation by protein kinases and dephosphorylation by protein phosphatases. Therefore, to investigate the regulatory mechanisms of signal transduction by protein phosphorylation, it is important to develop techniques for detecting and analyzing both protein kinases and protein phosphatases. In the present study, we developed a method for the detection of phosphatase activity using fluorogenic substrate, 4-methylumbelliferyl phosphate (MUP), after polyacrylamide gel electrophoresis. When rat tissue extracts were analyzed by the in-gel phosphatase assay using MUP, various phosphatases with different mobilities on Native-PAGE could be detected. We also analyzed phosphatases in the extracts from various developmental stages of mushroom, *Coprinopsis cinerea*. In this experiment, changes in the phosphatase activities during the developmental stages in *C. cinerea* were detected. Furthermore, we developed an in-gel phosphatase assay after separating proteins by a two-dimensional electrophoresis. The new two-dimensional electrophoresis consists of an isoelectric focusing using a MicroRotofor under non-denaturing conditions in the first dimension and Native-PAGE in the second dimension. Using the new in-gel assay after the non-denaturing two-dimensional gel electrophoresis, we could detect the changes in the activities of various phosphatases in crude cell lysates under varying conditions. Consequently, the present in-gel phosphatase assay will be applicable to the proteomic studies focused upon the phosphatases involved in various cellular functions or pathogenesis of various diseases.

P06-85
HCV NS5A protein containing potential ligands for both Src homology 2 and 3 domains enhances autophosphorylation of Src family kinase Fyn in B cells

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Hepatitis C virus (HCV) infects B lymphocytes and induces mixed cryoglobulinemia and B cell non-Hodgkin's lymphoma. The molecular mechanism for the pathogenesis of HCV infection-mediated B cell disorders remains obscure. To identify the possible role for HCV nonstructural 5A (NS5A) protein in B cells, we generated the stable B cell lines expressing Myc-His tagged NS5A. Immunoprecipitation study indicated that NS5A was tyrosine phosphorylated by pervanadate (PV) treatment of the cells. Therefore we examined pull-down assay by using glutathione S-transferase (GST)-fusion proteins of various Src homol-

ogy 2 (SH2) domains, which associates with phosphotyrosine within a specific amino acid sequence. The results showed that NS5A specifically bound to SH2 domain of Fyn from PV-treated B cells in addition to Src homology 3 (SH3) domain. Substitution of Arg176 to Lys in the SH2 domain of Fyn abrogated this interaction. Deletion mutational analysis demonstrated that N-terminal region of NS5A was not required for the interaction with the SH2 domain of Fyn. Far-western analysis revealed that SH2 domain of Fyn directly bound to NS5A. Fyn and NS5A were co-localized in the plasma membrane. These results suggest that NS5A directly binds to the SH2 domain of Fyn in a tyrosine phosphorylation-dependent manner. Lastly, we showed that the expression of NS5A in B cells increased in phosphorylation of activation loop tyrosine in the kinase domain of Fyn. Conclusion: NS5A containing ligand for both SH2 and SH3 domains enhances an aberrant autophosphorylation of Fyn kinase in B cells.

P06r-86
Sam68 expression and phosphorylation is increased by leptin and insulin in human breast adenocarcinoma cells

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Introduction: Obesity is a well known risk factor for breast cancer development in postmenopausal women. High insulin levels, together with other hormones, such as leptin and cytokines, IGFs, estrogen and EGF, positively modulate the growth of these tumor cells. All these factors may act through signaling cascades that lead to the final effect of increasing growth and cell proliferation. Sam68 protein is a member of the signal transduction activator of RNA (STAR) family of RNA-binding proteins that can interact both with RNA and signalling proteins. According to this dual role, Sam68 has been involved in different carcinogenic mechanisms. Moreover, our group has previously described the role of Sam68 in the insulin and leptin signaling pathways as a receptor substrate, and it has been shown to participate in proliferation, cellular growth and antiapoptotic effects mediated by these hormones in different cellular types.
Objective: We aim to study the expression of Sam68 and its phosphorylation level upon insulin and leptin stimulation, seeking for a possible role of Sam68 in leptin and insulin receptor signaling in human breast adenocarcinoma cells.
Methods: We used the human breast adenocarcinoma cell line MCF7. We studied leptin-mediated and also insulin-mediated Sam68 phosphorylation by immunoprecipitation and immunoblot with anti-phosphotyrosine antibodies. Quantitative RT-PCR and immunoblot were used to study the effect of leptin and insulin on Sam68 expression.
Results: Sam68 protein quantity and gene expression were found to be increased under leptin as well as insulin stimulation, by using 1 nM dose after a 24 hours stimulus. Moreover, both insulin and leptin stimulation promote an increase in Sam68 tyrosine phosphorylation in MCF7 cells, as previously observed in other systems.
Conclusions: These results suggest the participation of Sam68 in both leptin and insulin receptor signaling in human breast cancer cells, where Sam68 could mediate the trophic effects of these hormones in proliferation and cellular growth of non genetic breast cancer.

P06-87
The analysis of the mechanism by which mTOR complex 2 regulates actin cytoskeleton

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Mammalian target of rapamycin (mTOR) is a center protein that integrates the signals from nutrients, growth factors and energy. Two functional complexes containing mTOR, called mTORC1 and mTORC2, regulate many cellular events such as cell growth, cell size, autophagy, and actin cytoskeleton. However, the mechanism how mTORC2 regulates actin cytoskeleton remains to be elucidated. We first searched the proteins binding to an mTORC2 component rictor and identified two novel rictor binding proteins, filamin A and a kinesin family protein, as well as known mTORC2 components. In HeLa cells, rictor was concentrated in membrane ruffles with filamin A and F-actin. The accumulation of rictor was diminished by the removal of serum and recruited again by re-stimulation with insulin. In addition, insulin increased the phosphorylation of filamin A. The treatment of HeLa cells with mTOR catalytic inhibitor Torin1 suppressed this phosphorylation, accompanied by decreased cell migration. Our data suggest that mTORC2 modifies filamin A phosphorylation to regulate actin cytoskeleton.

P06-88
Adipose mesenchymal stem cells migration is stimulated by inflammatory conditions via fibronectin-dependent activation of PDGF receptors

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The migration of the mesenchymal stem cell (MSC) to the sites of tissue injury is an important part of tissue regeneration process; yet little is known about the chemotactic factors inducing MSC migration toward the injury. In the present study we tested the hypothesis that the migration of MSC to the injured tissues is driven by inflammatory milieu. To modulate inflammatory process we co-cultured MSCs and macrophages derived from THP-1 cell contactlessly and measured the migration rate of MSCs in a scratch-assay. We found that pro-inflammatroy factors secreted by THP-1 macrophages up regulate MSC migration more than two fold in comparison to MSC migration in the absence of THP-1. We also analyzed directional migration of MSCs using xCELLigence system (Roche) and found that THP-1 stimulate MSC-migration through the fibronectin-coated membrane. This effect was abrogated if the membranes were coated with collagen I. Importantly an inhibitors of PDGFRβ autophosphorylation (AG1296) and PI3K activation (LY294002) almost completely blocked the THP-1 stimulatory effects on MSCs migration. Correspondingly, the levels of EGFR and PDGFRβ protein were significantly increased. However, the level of phosphorylated Akt was significantly increased when inflammatory-conditions-treated MSCs were placed on fibronectin-coated plastic. Interestingly, we did not observed this effects if MSCs were cultured on the uncoated membranes. Co-culturing of MSCs with THP-1 cells also resulted in the increase of fibronectin-binding integrins α5β1 subunits α5 and β1. Thereby the activation of MSCs migration in inflammatory conditions is mediated by fibronectin binding and activation of PDGFRβ-dependent signaling pathways.

P06-89
Co-regulation of NF-kappaB sygnaling pathway by the active form of Heat Shock Factor 1

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NFκB- and HSF1-dependent pathways play the important role in response to therapeutic treatments and pathogenesis of serious human diseases, including cancer. Both of these transcription factors regulate several genes involved in apoptosis, cell proliferation and inflammatory responses. Here we aimed to identify NFκB-dependent genes which expression is affected by the active HSF1. Activation of the NFκB pathway and expression of NFκB-dependent genes was analyzed in U2-OS human osteosarcoma cells stimulated with TNFα cytokine. Cells were either preconditioned with hyperthermia to activate endogenous HSF1 (*wt*) or engineered to express a constitutively active form of HSF1 in the absence of heat shock (*tg*). The expression of NFκB-dependent genes was analyzed by microarrays and quantitative RT-PCR. Binding of HSF1 to promoters of NFκB-dependent genes was analyzed by ChIP assay with anti-HSF1 Ab. We have found that the presence of constitutively active HSF1 did not block TNFα-induced activation of the NFκB pathway, but affected expression of some NFκB-dependent genes in the absence of the heat shock. Four of these genes, namely TNFA, IL-6, FASLG, AGT, contained functional binding sites for HSF1 in their promoter regions. Additionally, microarrays-based analysis revealed that expression of certain NFκB-dependent genes was up-regulated without TNFα stimulation in heat shocked *wt* cells and control *tg* cells. We concluded that expression of several NFκB-dependent genes is modulated by HSF1-dependent mechanisms. Some of these genes could be directly co-regulated by HSF1 due to the binding of this transcription factor to promoter regions of such genes. This work was supported by the Ministry of Science and Higher Education, grants N N401 563740 and N N518 287540.

P06-90
TCTEX1D4 interactome: relevance for male reproductive function

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T-complex testis expressed protein 1 domain containing 4 (TCTEX1D4) is a dynein light chain of the DYNLT1/TCTEX1 family that was identified as a PhosphoProtein Phosphatase 1 (PPP1) interacting partner in human testis. Localization in sperm of TCTEX1D4 and PPP1CC2, a testis-enriched and sperm-specific PPP1 isoform, suggests a role of this holoenzyme in sperm motility, intraflagellar transport and/or acrosome reaction. In cultured cells, TCTEX1D4 localization appears to regulate the retrograde transport of PPP1 to specific subcellular regions. The specific functions of TCTEX1D4 in testis are still unknown. A yeast two hybrid approach was undertook to identify TCTEX1D4 interactome with the goal of clarifing TCTEX1D4 function in testis. 2.59×10^6 clones were screened resulting in 494 positive clones from which 86 clones were already identified corresponding to 44 different proteins. The interactomes of the 44 proteins were retrieved from public databases and a protein network was constructed using Cytoscape. Also, seven protein subnetworks were constructed corresponding to the TCTEX1D4 interacting proteins which are testis specific or enriched and TCTEX1D2, a known flagellar protein with the purpose of identifing specific testis protein complexes. CRISP2 and TCTEX1D2 were further studied: CRISP2 is present in testis and appears to mediate the interaction of spermatogenic cells with Sertoli cells and in sperm as an intra-acrosomal protein present in the principal piece of the tail; TCTEX1D2 is a dynein light chain protein and was first identified in *Chlamydomonas* as part of the outer dynein flagellar arm. The results presented will shed light on TCTEX1D4 protein complexes and their putative functions in human testis and spermatozoa, with special focus in CRISP2 and TCTEX1D2.

P06-91
Prolyl hydroxylase-dependent modulation of eukaryotic elongation factor 2 activity and protein translation under acute hipoxia

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Hypoxia is a physiologic condition associated with different pathologies. Therefore, adaptive responses to decreased oxygen availability are essential for cell survival. Transcriptional changes have been a matter of study for long time, however the acute post-transcriptional changes elicited by hypoxia are poorly known. We have studied the differences between normoxia and acute hypoxia in mammalian cells using a proteomic approach. We have found that phosphorylation of eukaryotic elongation factor 2 (eEF2), a ribosomal translocase whose phosphorylation inhibits protein synthesis, is under precise and reversible control of O_2 tension. eEF2 phosphorylation at Thr^{56} occurs rapidly (<15 min) and is independent of AMP kinase activation, a enzyme involved in translational arrest under ischemic conditions. As a consequence, the protein synthesis rate is slow down leading to a modest reduction in the rate of ATP consumption, a fundamental homeostatic response that promotes cell survival. Phosphorylation of eEF2 is mimicked by pharmacological (DMOG) or genetic (siRNA) prolyl hydroxylases (PHDs) inhibition. eEF2 phosphorylation is controlled by PHDs but is independent of hypoxia inducible factor α (HIFα) stabilization. Furthermore, the over-expression of PHDs in hypoxia blocks hypoxic accumulation of phosphorylated eEF2. All these results suggest that the cellular oxygen sensors PHDs control eEF2 phosphorylation status and translational rate.

P06-92
Trafficking and functional coupling properties of Melanocortin 1 receptor-Tubulin-beta-III intergenic splice variants

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The *melanocortin 1 receptor* gene (*MC1R*) expressed in epidermal melanocytes is a major determinant of constitutive and ultraviolet radiation (UVR)-induced human skin pigmentation. It encodes a Gs protein-coupled receptor activated by α-melanocyte stimulating hormone (αMSH). Signaling from MC1R triggers the

cAMP pathway and the cAMP-independent activation of extracellular signal regulated protein kinases ERK1 and ERK2. Human *MC1R* has a very inefficient poly(A) site which allows intergenic splicing with its inmediate downstream neighbour *Tubulin-β-III* (*TUBB3*). Intergenic splicing yields 2 MC1R isoforms bearing the extracellular N terminus and the seven transmembrane helices from MC1R fused to TUBB3-derived C-terminal extensions, in-frame for Iso1-MC1R and out-of-frame for Iso2-MC1R. Treatment of melanoma cells with key molecules associated with UVR such as αMSH, or activation of p38-MAPK promotes expression of MC1R-TUBB3 chimeras, which might lead to novel phenotypes required for tanning.

We analyzed the functional and trafficking properties of the intergenic splicing isoforms. In heterologous HEK cells, Iso1 was expressed as a single band of the expected size, whereas Iso2 yielded a doublet of Mr lower than predicted and low intracellular stability. In equilibrium binding experiments, Iso1- and Iso2-MC1R displayed an affinity similar to WT-MC1R, but a reduced cell membrane density. Analysis of internalization rates and confocal microscopy indicated that the low cell surface expression of the isoforms was mostly associated with aberrant forward trafficking with extensive intracellular retention in the endopalsmic reticulum. Functional coupling to cAMP was also impaired, but ERK activation upon binding of αMSH was similar than WT, indicating imbalanced signaling from the splice variants. Heterodimerization of differentially labeled MC1R and the isoforms was efficient and fucntionaly relevant as it decreased surface expression of binding sites. Thus, the MC1R isoforms induced by UVR might contribute to the regulation of the tanning response by a reduction of MC1R availability and a modulation of its functional parameters.

P06-93
NGF regulates EGF, NGF and insulin at the expression, synthesis and secretion levels in beta cells of rats

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Insulin is stored in secretory granules in pancreatic β cells and released by exocytosis upon stimulation with several different stimulators such as glucose. More recently, some growth factors such as EGF and NGF have been characterized as potent insulin secretagogues. Although the mechanism of growth factor-induced insulin secretion was investigated, the relation of these growth factors in this process is still unknown. The aim of this study was to investigate whether nerve growth factor (NGF) has an effect on the synthesis and secretion of epidermal growth factor (EGF), insulin and NGF of β cells isolated from normoglycemic and hyperglycemic rats. To answer these questions, we injected streptozotocin (STZ), which induces hyperglycemia by destroying pancreatic β cells, to rats. β cells were isolated and treated with NGF neutralization antibody for 24 hours to withdraw NGF in culture medium. The following results were noted by NGF neutralization: (i) down-regulation of insulin gene expression, while up-regulation of NGF and EGF gene expressions were observed in β cells. (ii) insulin protein level was decreased, while NGF and EGF protein levels were increased in the β cells; (iii) NGF, EGF and insulin secretion from β cells of were decreased. Although the results look like similar in euglycemic and hyperglycemic conditions, NGF withdrawal more strictly affected β cells from hyperglycemic rats. The data presented here are the first proofs suggesting that NGF regulates EGF, NGF and insulin at the expression, synthesis and secretion levels in β cells of rats.

P06-94
Roles of calcium/calmodulin-dependent protein kinase I-delta (CaMKI-delta) isoforms in the early embryogenesis of zebrafish

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CaMKI-delta (CaMKId) is known to be expressed ubiquitously in various tissues, but little is known about the functions during early embryogenesis. To investigate the biological significance of CaMKId during zebrafish embryogenesis, we isolated cDNA clones for CaMKId isoforms by expression screening using cDNA library from embryos at 72-hours post-fertilization (hpf). There are two splice variants with different C-terminal sequences, comprising of 392 and 368 amino acids, and they are designated CaMKId-L and CaMKId-S, respectively. Furthermore, we cloned CaMKId-LL, a new CaMKId isoform of 433 amino acids, located on a different chromosome from CaMKId-S/L. Although the catalytic domain of CaMKId-LL showed 86% identity with that of CaMKId-S/L, it had a unique C-terminal sequence. All of these CaMKId isoforms were activated when they were phosphorylated by CaMK-kinase. Activated CaMKId significantly phosphorylated various proteins including CREB, histones, MLC and MBP. RT-PCR analysis revealed that mRNAs of CaMKId-LL and CaMKId-S clearly appeared after 48 and 72 hpf, respectively. On the other hand, the transcript of CaMKId-L was detectable in just-fertilized eggs (0 hpf), and it disappeared at 12 hpf, and appeared again at 24 hpf and thereafter. Western blotting analysis in adult fish demonstrated wide distribution of CaMKId-LL in the brain, eyes and fin. In contrast, CaMKId-S was specifically expressed in the brain, while CaMKId-L was presented in the brain, eyes, ovary and especially abundant in skeletal muscle. The knockdown of CaMKId-S/L with morpholino-based antisense oligonucleotides resulted in an increase of abnormal embryos with short trunk, kinked tail and small heads. In contrast, the knockdown of CaMKId-LL resulted in an increase of abnormal embryos with small fin. These phenotypes were rescued by coinjection with recombinant CaMKId but not with their kinase-dead mutants. These results clearly indicate that the kinase activities of CaMKId isoforms play crucial roles in the early embryogenesis of zebrafish.

P06-95
Anandamide activates p-38 pathway in decidual cells

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The main active component of *Cannabis sativa*, Δ9-tetrahydrocannabinol, exerts its pharmacological effects by binding to selective membrane receptors, which are also activated by a endogenous family of lipid messengers, called endocannabinoids (ECs). Prior studies evidenced that ECs are involved in the regulation of a variety of cell-signalling pathways. We have previously demonstrated that ECs machinery operates on decidual cells and found that AEA, the principal EC, induced apoptosis in decidual cells through cannabinoid receptor 1 (CB1). CB1 is a member of the superfamily of G-protein-coupled receptors, which inhibit adenylyl cyclases, regulate ionic channels and modulate the activation of mitogen-activated protein kinases (MAPK). Additionally, we found that AEA induces an increase in ceramide levels, effect dependent on CB1 activation. In the present study we intend to investigate the signal transduction pathways of AEA in rat primary decidual cell cultures by comparing the effects of isolated ceramide and dihydroceramide, as well as, by

using inhibitors for MAPK's transduction pathways. Our data provide evidences that as AEA, ceramide induces phosphorylation of p38, but not dihydroceramide. In addition, the pre-treatment with the specific inhibitor for p38, the SB203580 reverted the reduced cell viability effects induced by AEA. Collectively, in primary decidual cells, AEA induced an increase in ceramide levels which may evoke p38 phosphorylation. More studies are required to understand the exact biochemical mechanisms underlying the decidual regression process and the involvement of AEA in activation of p38 pathway mediated by ceramide, which subsequently may result in apoptosis.

P06r-96
Regulation of mitotic exit by cell cycle checkpoints

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During cell division, it is essential to ensure that the daughter cells receive a whole set of the genome. Problems with the segregation of chromosomes lead to the generation of cells with an abnormal DNA content. Aneuploidy is a condition that is defined by an alteration of the normal karyotype of a cell so that the chromosome number is not an exact multiple of a haploid content. This condition is a hallmark of cancer and a number of different diseases. In *Saccharomyces cerevisiae*, three different surveillance mechanisms maintain the fidelity of the transmission of the genetic information during the cell cycle and impede the generation of aneuploid products: the DNA damage, the spindle assembly (SAC) and the spindle position (SPOC) checkpoints. These surveillance mechanisms block cell cycle progression by restraining mitotic exit, the final transition of the cell cycle. The regulation of mitotic exit by these checkpoints is mediated by Bfa1 and Bub2, a two-component GAP (GTPase activating protein) that negatively regulates the Tem1 GTPase. Tem1 initiates a Ras-like signalling cascade called the mitotic exit network (MEN), which triggers mitotic exit in budding yeast. The MEN maintains a sustained release of the phosphatase Cdc14 from the nucleolus during anaphase and determines mitotic exit by inactivating CDK activity. Our work provides new insights into how the different checkpoints that maintain genome integrity regulate Bfa1 and Bub2 to inhibit mitotic exit and provide the cells with time to fix errors during the distribution of the genomic material.

P06-97
Quantitative real-time PCR study of the inhibitory activity of organic acids produced by a probiotic culture on Staphylococcus aureus quorum sensing and virulence genes expression

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Objectives: The purpose of this study was to demonstrate by real-time RT-qPCR the inhibitory activity of the sub-inhibitory (sI) concentrations of lactic and acetic acid, two antimicrobial compounds produced by *Lactobacillus paracasei* subsp. *paracasei* cultures, on the expression level of virulence and quorum-sensing (QS) genes in *Staphylococcus (S.) aureus* multidrug resistant strains. These acids have been previoulsy identifies in the probiotic culture filtrate, quantified by capillary electrophoresis – diode array detection, and tested for their inhibitory activity on *S. aureus* enzymatic virulence factors expression by phenotypic assays.
Methods: The study was performed on 10 *S. aureus* strains isolated from cardiovascular devices associated infections. Total RNA was extracted from control (*S. aureus* strains grown in standard conditions) as well as from samples (*S. aureus* strains grown in the presence of sI concentrations of lactic and acetic acid). For the quantification of*agr* gene expression there was used real-time RT-qPCR with minor groove binder probes with a non-fluorescent quencher bound to the 3′end, whereas for the quantification of virulence genes expression involved in invasion and toxigenesis, that are regulated by agr QS system, we used RT-qPCR with SYBR-Green.
Results: The results of qRT-PCR have shown that in all *S. aureus* strains grown in the presence of sI concentrations of organic acids, the level of QS and virulence genes expression was reduced comparatively with those from control cultures.
Conclusion: These results proved that the inhibition of virulence factors regulation mechanisms by organic acids secreted by probiotics could contribute to the pathogenicity and virulence attenuation in *S. aureus* strains isolated from cardiovascular devices associated infections.
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P06-98
Functional interactions of human melanocortin 1 receptor, cytosolic arrestin isoforms and the E3 ubiquitin ligase Mahogunin Ring Finger 1

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MC1R signaling is crucial for melanocyte proliferation and differentiation, and *MC1R* mutations are associated with skin tanning defects and increased melanoma risk. MC1R is a G protein-coupled receptor whose interaction with the transducing Gs protein is modulated by members of the β-arrestin (ARRB) family of cytosolic proteins which mediate signal termination and endocytosis of receptor-agonist complexes, as well as by the RING domain E3 ubiquitin ligase Mahogunin Ring Finger-1 (MGRN1), whose mutation increases pigmentation and leads to neurodegeneration. We have analyzed the interactions of ARRBs, MGRN1 and MC1R. We found agonist-independent competitive interactions of ARRB1 and ARRB2 with MC1R. Although both ARRBs engaged MC1R-ARRB associations independently on phosphorylation of MC1R C-terminal Ser/Thr residues, only ARRB2 triggered receptor internalization and signal termination. A stable MC1R-MGRN1 association was also detected by co-immunoprecipitation and gel filtration analysis. This interaction might involve the RING finger-containing C-terminal domain of MGRN1. Co-expression of MC1R with the ARRBs caused a strong increase in ARRB ubiquitylation, even in the absence of MC1R agonists. ARRB ubiquitylation was nevertheless dependent on correct cell surface expression of MC1R, as well as on its native conformation, since MC1R-dependent ub-

iquitylation was lower or absent for MC1R mutants with extensive intracellular retention or unable to undergo transition to an active state. Importantly, MGRN1 did not trigger ARRB ubiquitylation in the absence of MC1R, but MC1R-dependent ARRB ubiquitylation increased markedly on co-expression of MGRN1, and decreased upon specific silencing of the E3 ligase. These data increase the repertoire of signaling events downstream of MC1R by incorporating receptor-dependent ARRB ubiquitylation and suggest that MGRN1 might function as a melanocytic MC1R-dependent ARRB ubiquitin ligase.

P06-99
Soluble Heparin-binding EGF-like growth factor promotes EGF receptor nuclear translocation

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Heparin-binding EGF-like growth factor (HB-EGF) is a member of the EGF family of growth factors. It has high affinity for heparin and heparan sulfate. HB-EGF precursor is cleaved by proteases yielding soluble HB-EGF (sHB-EGF). sHB-EGF mediates paracrine and autocrine activation of the EGFR family members: EGFR and ErbB4 and thereby promotes survival, proliferation and migration in different cell types. Ligand binding to EGFR results in EGFR activation and further recycling or lysosomal degradation of ligand-receptor complex. In nucleus EGFR acts as transcription factor activating genes required for proliferating activities. EGFR ligands: EGF, TGF-α and heparin-binding amphiregulin have all been reported to accumulate in the nucleus in several cell types. EGF and amphiregulin were localized in the nucleoli as soluble form of factors but TGF-α as a precursor. Nevertheless there is no evidence on the nuclear localization of sHB-EGF and its ability to induce EGFR nuclear importation after binding. We demonstrated sHB-EGF induced EGF receptor nuclear importation via retrograde transport. By chromatin immunoprecipitation assay we showed the association of sHB-EGF-induced nuclear EGFR with promoter region of cyclin D1 in A431 cells. Moreover, we demonstrated that the translocation complex consisted of both ligand and receptor. It was also shown that addition of EGFR kinase inhibitor – AG1478 dramatically decreased sHB-EGF-induced EGFR internalization and further nuclear translocation of ligand-receptor complex, which means that EGFR kinase plays an important role in induction of intracellular internalization process. Therefore sHB-EGF might function as an extracellular inductor of EGFR nuclear importation to activate genes required for highly proliferating activities.

P06-100
Phosphoinositide-dependent protein kinase-1 (PDK1) ortholog Ksg1 regulates cell wall integrity MAPK pathway in the fission yeast Schizosaccharomyces pombe

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Ksg1, an essential gene, is the ortholog of the mammalian phosphoinositide-dependent protein kinase-1 (PDK1) in *Schizosaccharomyces pombe*. Previous studies in animal cells have established that PDK1 phosphorylates and activates Protein Kinase C Related kinases (PRKs) and several Protein Kinase C isoforms (PKCs) in a conserved domain in their activation loop. Interestingly, PDK1 dependent phosphorylation on these substrates requires a previous conformational change mediated by their interaction with RhoA GTPase. Two hybrid studies have shown that Ksg1 interacts with both Pck1 and Pck2, the two PKC orthologs in fission yeast, and this association is important for cell wall integrity control (Graub *et al*., 2003). However, the precise mechanism/s involved in Ksg1 regulation of both kinases remains obscure. Recent data have demonstrated the critical role of Rho1 and Rho2 GTPases, together with Pck2 and Pck1, in the regulation of the cell integrity MAP kinase pathway, whose central element is MAPK Pmk1. This kinase becomes activated in response to multiple stressing situations and during cell separation (Ma *et al*., 2006, Barba *et al*., 2008, Cansado *et al*., unpublished results). Notably, the identity of the elements operating upstream of the MAP kinase module during signal transmission depends on the nature of the stimulus (Barba *et al*., 2008).

In this work we have employed Pmk1 as a biological reporter to unravel how Ksg1 regulates PKCs in fission yeast and its role during cell integrity control. We show that Pmk1 activation in response to hyperosmotic stress, which is channeled through the Rho2-Pck2 branch (Barba *et al*., 2008), and cell wall stress, which is dependent on both Rho1 and Rho2 GTPases (Cansado *et al*., unpublished results), is fully dependent on Ksg1 activity. Moreover, Pmk1 hyperactivation induced by overexpression of Rho1 and Rho2 GTPases is compromised in *ksg1* thermosensitive mutants, suggesting that Rho-PKC signaling to the MAPK module requires previous activation by Ksg1. Finally, we will address the molecular mechanism by which Ksg1 regulates PKCs and/or RhoGTPases in the context of cell wall integrity signaling.

P06r-101
The E3 ubiquitin ligase HERC2 regulates p53 stability and signaling

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Activation of p53 coordinates the cellular response to several types and levels of stress, regulating a broad range of genes involved in apoptosis, cell cycle arrest, senescence, DNA repair, cell metabolism or autophagy. The activation of p53 is a process highly regulated that includes post-translational modifications such as ubiquitination, phosphorylation and acetylation or protein-p53 interactions. Here, we report that the E3 ubiquitin ligase HERC2 interacts with p53 in different human cell lines. This interaction involves the CPH domain of HERC2 and the last 43 amino acid residues of p53 that contains part of the tetramerization domain of p53. HERC2 depletion destabilized p53 and other proteins regulated by p53 such as p21. The interaction HERC2-p53 is disrupted by p53 mutants in the tetramerization domain which cannot form oligomers. Additionally, we demonstrated that this interaction occurs predominantly in the cytoplasm, a nucleus-confined p53 mutant cannot associate with HERC2. Knockdown of HERC2 increases the cellular growth and the focus formation in clonogenic assays. These findings demonstrate HERC2 as a regulator on p53 stability and p53-mediated cellular processes.

P06r-102
Loss of Sprouty1 accelerates PTEN-induced tumor formation by suppressing cellular senescence

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Genes of the Sprouty family are regarded as inhibitors of receptor tyrosine kinase, especially of the ERK MAPK pathway. As such, they have been proposed as candidate tumor suppressor genes in a wide variety of cancerous pathologies. However, genetic evidence supporting a causal link between loss of Sprouty function and tumor development is missing. In this work we aimed to analyze tumor development in Spry1 knockout mice. Spry1 knockout mice up to five months of age present enlarged thyroids and lymphoadenopathy but no signs of tumoral growth. Analysis of thyroid glands demonstrate that loss of Spry1 results in increased proliferation of follicular cells, which is not caused by either elevation of systemic TSH levels or increased activation of the ERK MAPK pathway. In contrast, thyroids from Spry1 knockout mice appear to escape cellular senescence, a potent barrier to tumoral transformation. To assess whether loss of Spry1 might accelerate the onset of neoplastic transformation in a tumor-prone mouse, we crossed Spry1 knockout mice to PTEN heterozygous mice, which develop carcinomas of the thyroid gland, among others. Double mutant mice show increased incidence of tumors of the thyroid, adrenal glands and lymphoid tissues when compared to $PTEN^{+/-}$ mice. Thus, we propose that Spry1 contributes to tumor suppression by inducing cellular senescence.

P06r-103
Regulation of SHP1 oxidation regulates megakaryocytic differentiation in HEL cells

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The production of reactive oxygen species (ROS) has traditionally been related to deleterious effects for cells. However, it is now widely accepted that ROS can play an important role in regulating cellular signalling and gene expression. NADPH oxidase ROS production seems to be especially important in this regard. Our team has recently shown that NADPH oxidase driven ROS production is required for megakaryocytic differentiation. It is thought that one of the possible ROS targets are protein tyrosine phosphatases (PTPs), because their activity is very sensitive to oxidation. Therefore, we have started to study if this is the case in our system, focusing in SHP1, a PTP that is clearly involved in haematopoiesis. Our data show that SHP1 is important for megakaryocytic differentiation because whenwe reduce the level of SHP1 by iRNA, megakaryocytic markers levels are increased, and when we increase SHP1 level megakaryocytic markers levels are decreased. Therefore, it seems feasible that the regulation of SHP1 activity could be important for megakaryopoiesis. In this regard, our data show that SHP1 activity suffers a sharp decrease at the beginning of megakaryocytic differentiation. This decrease is partially avoided when we add DPI, a NADPH oxidase inhibitor. Moreover, when we look at the oxidation of the cysteine residue required for catalysis, we find that SHP1 oxidation also increases during the first minutes of the megakaryocytic differentiation, and this oxidation is also partially avoided by DPI treatment. These data suggest that SHP1 oxidation and inhibition by NADPH oxidase driven ROS production might be a crucial event in the regulation of megakaryocytic differentiation. Ministerio de Economía y Competitividad Junta de Castilla y León

P06-104
Regulatory role of the M3/6 dual-specificity phosphatase in JNK signaling

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Signal transduction by the c-Jun N-terminal Kinase (JNK) is important in the regulation of diverse cellular processes. The JNK family consists of at least ten different splice variants coded by three distinct JNK genes. While some functional differences between JNK isoforms have been demonstrated, little is known about the underlying molecular mechanisms and the specific roles of individual splice variants. Specific signaling outputs by JNK family members depend on the intensity and duration of their activation. Therefore, phosphatases play a crucial role in these events, by tightly regulating JNK kinase activity. M3/6 is a dual specificity phosphatase (DUSP) with an established role in the dephosphorylation and inactivation of JNK, and to a lesser extent p38. We have investigated the interaction of M3/6 with distinct JNK isoforms, as well as with scaffold proteins of the JIP family, in order to elucidate the contribution of M3/6 to the regulation of JNK isoform signaling specificity. M3/6 showed high affinity towards JNK1b and JNK 2a isoforms and this was reflected in enhanced enzymatic activity towards JNK2a2, when compared to JNK1a1 *in vitro*. Following treatment of 293T cells with arsenite, the interaction of M3/6 with JNK1α and JNK3 was enhanced, while that with JNK1β/2α decreased. The interaction of M3/6 with JIP1 or JIP2 scaffold proteins was constitutive, whereas arsenite treatment induced the recruitment of M3/6 to JIP3 complexes. We have additionally used a proteomic approach using biotin-tagging to affinity-purify proteins interacting with M3/6 and thus gain a better understanding of the regulatory mechanisms governing M3/6 activity towards its substrates. Preliminary data from these experiments indicate that proteins regulating oxidative stress responses are enriched in M3/6 pull-downs. Taken together, our data suggest a specific targeting of M3/6 towards distinct JNK-containing signaling complexes. This work was supported by the European Commission FP7 program INFLA-CARE (EC contract number 223151).

P06-105
TORC1 mediates loss of mitochondrial function in isc1Δ cells

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The Tor signaling is known to be conserved among organisms, ranging from budding yeast, fruit flies and higher eukaryotes. In the budding yeast *Saccharomyces cerevisiae*, TOR signaling is mediated by two PI3-kinase-like serine-kinases encoded by the *TOR1* and *TOR2* genes. The rapamycin-sensitive TORC1 branch regulates important cellular processes, namely ribosome biogenesis, autophagy and cell growth and more recently it was implicated in the regulation of yeast chronological lifespan (CLS) by modulating mitochondrial function and mitochondrial ROS

(reactive oxygen species) production. Sphingolipids, as ubiquitous components of eukaryotic membranes, play crucial roles as signaling mediators by regulation important biological processes, such as stress response, cell growth and apoptosis/cell survival. However, how sphingolipids and TORC1 signaling cooperate in lifespan and mitochondrial function regulation remains unknown. In *Saccharomyces cerevisiae*, ceramide can be produced by the *novo synthesis* pathway or generated by the hydrolysis of complex sphingolipids by a process catalysed by the inositiol phosphosphingolipid phospholipase C protein, Isc1p, the yeast orthologue of the mammalian neutral sphingomyelinase 2. Cells lacking Isc1p display shortened CLS, oxidative stress sensitivty and mitochondrial dysfunctions associated with ceramide-mediated activation of Sit4p protein phosphatase and Hog1p MAP kinase. In this study, we show that the deletion of the *TOR1* gene abolished the premature aging, hydrogen peroxide sensitivity, loss of mitochondrial membrane potential, respiratory defects, increased ROS production and catalase A deficiency of *isc1*Δ cells. The overall results suggest that TORC1 functions downstream of Isc1p and implicate TORC1 on mitochondrial dysfunction, premature aging and oxidative stress sensitivity of *isc1*Δ cells.

P06-106
Metformin induces pancreatic beta cell apoptosis and modulates TSC2-SIRT1 interaction

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One of the more widespread treatments for type 2 diabetics is metformin. Metformin acts primarily on liver, inhibiting gluconeogenesis. It is also known that metformin is able to block ATP production. Is thought to act partly by the activation of AMP-activated protein kinase (AMPK) and very recently it has been proposed AMPK-independent effects. Furthermore, metformin can exert its actions by TSC2-dependent and independent mechanisms. Then, it is essential to delineate the underlying molecular mechanism of biguanides at a molecular level for a better understanding of drug action. In addition, TSC2 interacts with different proteins and this interaction can be modulated in response to different stimuli. Apart from TSC2 phosphorylation in different residues, essential for mTORC1 activation or inhibition, TSC2 activity can be modulated by other posttranslational modifications. TSC2 acetylation and its role on mTORC1 activity are very poorly studied. To study the effect of metformin we have used several pancreatic β cell lines compared with different non-pancreatic cell lines. Furthermore, we have used different AMPK constructs (AMPK WT, AMPK K45R and AMPK T172D) or compound C for understanding the AMPK-dependent and independent effects of metformin on pancreatic β cell apoptosis. We have generated different β cell lines with TSC2 deletion by shRNA (MIN6, INS1E) for study the role of TSC2 in metformin action. We have observed that metformin (0.5–2 mM) induced cell apoptosis in pancreatic β cell lines, while non-pancreatic β cell lines were resistant to metformin-induced cell death at higher doses (fragmented nuclei, haplodiploid phase, annexin V positive cells, caspase-3 activation). Metformin-induced pancreatic β cell death is partly inhibited by the use of AMPK inhibitors (compound C and AMPK K45R). TSC2 is associated with the NAD-deacetylase SIRT1 under basal conditions. Furthermore, this interaction is disrupted under different energetic stressors such as metformin, 2DG and glucose deprivation. In addition, this interaction modulates the acetylation degree observed in TSC2. Here, we show that metformin present a pro-apoptotic effect, exclusively in pancreatic β cells. In addition, we proposed that TSC2 acetylation is important for mTORC1 regulation under different cellular situations.

P06-107
Role of PPAR signaling in the cancer progression of human prostate cancer

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Prostate cancer remains one of the major cause of cancer-related mortality in men. Peroxisome proliferator-activated receptor gamma (PPAR≥) ligands seem to induce anticancer effects on different cancer cells, but the mechanism is not clear. PPARs also exert several vascular effects that may provide a dual benefit of these receptors on metabolic disorders and cancer. We recently reported that PPAR≥ ligand ciglitazone led to reduction of proliferation and stimulation of differentiation of prostate cancer cells through the increased expression or reexpression of E-cadherin with parallel inhibition of N-cadherin expression and down-regulation of β-catenin. The effect depends on type of the cells and hormone dependency. PPAR≥ has been shown to regulate phosphatidylinositol 3-kinase (PI3K) signaling by modulating the expression of phosphatase and tensin homologue deleted on chromosome ten (PTEN). It is known that activation of PI3K/Akt pathway leads to suppression of apoptosis and may among other stabilize β-catenin through inhibition of GSK-3β by its phosphorylation at S9 and/or direct phosphorylation of β-catenin S552 or 675 resulting in its nuclear accumulation. The synthetic PPAR≥ ligand rosiglitazone significantly reduced expression of PI3K/Ak 473 and nuclear β-catenin S552 as well as inhibited migration due to reduced expression of MMPs and angiogenesis.

VEGF-induced activation of Akt is completely blocked by treatment with a specific PI3K inhibitors as LY294002 or type X. They reversed the ability of VEGF to promote cancer progression. Additionally the other inhibitor FH535 blocks β-catenin/PPARγ interaction. These results provide evidence for the antimigratory effects of PPARγ-activators and may also protect the vasculature associated with cancer progression.

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P06-108
Nucleotides released from breast cancer cells MDA-MB-231 increase proliferation and invasion through $P2Y_2$ receptor activation

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Extracellular nucleotides are released from injured or stressed cells and tissues whereupon they activate cell surface P2 receptors

belonging to two structurally distinct families, the G protein-coupled P2Y receptors (P2YRs) and the ion channel P2X receptors (P2Xs). It is reported that $P2Y_2R$ mediates proinflammatory responses such as cell migration and proliferation. However, the role of $P2Y_2R$ on cancer metastasis remains unclear. Thus, this study aimed to determine the role of $P2Y_2R$ on the proliferation, migration, and invasion of high metastatic breast cancer cell line MDA-MB-231, and on the interaction between breast cancer cells MDA-MB-231 and endothelial cells (ECs). Results showed that $P2Y_2R$ is expressed in both MDA-MB-231 and ECs, and that $P2Y_2R$ activation by ATP or UTP increased MDA-MB-231 proliferation, migration and invasion. Furthermore, ATP or UTP increased the expression of ICAM-1 and VCAM-1 resulting in increase of adhesion of MDA-MB-231 to ECs. Knock-down or inactivation of $P2Y_2R$ using siRNA or antagonist abolished the effect of ATP or UTP on cancer cell proliferation, migration, invasion and adhesion to ECs. Moreover, $P2Y_2R$ activation by ATP or UTP caused in increase of ECs' permeability through phosphorylation of VE-cadherin and decrease in VE-cadherin level in ECs. This study suggests that $P2Y_2R$ may play an important role in cancer metastasis through modulation of the crosstalk between cancer cells and ECs.

Key words: adhesion molecules, breast cancer cells, endothelial cells, metastasis, P2Y2 nucleotide receptor

P06-109
Effect of CCN2/CTGF on FGF2-induced proliferation of and MMP-9 and -13 productions by chondrocytes

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CCN2 (also known as connective tissue growth factor: CTGF) interacts with several growth factors involved in endochondral ossification via its characteristic 4 modules and modifies the effect of such growth factors. Presently we investigated whether or not CCN2 interacts with fibroblast growth factor 2 (FGF2). Solid-phase binding assay, immunoprecipitation-Western blot analysis, and surface plasmon resonance (SPR) spectroscopy revealed that the C-terminal module of CCN2 (CT) directly bound to FGF2 with a dissociation constant (K_d) of 5.5 nM. Next, we examined the combinational effects of CCN2 and FGF2 on the proliferation of and matrix metalloproteinase (MMP)-9 and -13 productions by cultured chondrocyets. FGF2 promoted not only the proliferation but also the production of MMP-9 and -13, however, CCN2 combined with FGF2 inhibited the MMP9 production by FGF2; and combination of the CT module and FGF2 nullified the enhancement of both MMP production and proliferation. To clarify the mechanism, we investigated the binding of CCN2 or its CT module to FGF receptor 1 (FGFR1). As a result, we found that CCN2 bound to FGFR1 with a K_d of 362 nM; whereas the CT module did not. In addition, when we tested FGF signaling in HCS-2/8 cells stimulated by the combination of CT module and FGF2, the level of ERK1/2, p38 MAPK, and JNK phosphorylation was decreased compared with that found with FGF2 alone. These findings suggest that CCN2 may regulate the proliferation and matrix degradation of chondrocytes by forming a complex with FGF2 as a novel modulator of FGF2 functions.

P06-110
Sodium tugstate increases protein synthesis in muscle cells through an activation of the mTOR signalling pathway

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Skeletal muscle hypertrophy is associated with an increase on protein synthesis; on the contrary, several disorders, as sepsis, AIDS or inactivity lead to muscle wasting leading to atrophy. The main mechanism that control muscle protein synthesis is the regulation of the initiation phase of translation through the phosphorylation of key elements of the mammalian target of rapamycin (mTOR) pathway. Sodium tungstate is an oral glucose-lowering and antiobesity agent. In L6 muscle cells, we observed that those cells differentiated in the presence of tungstate had an increase in the net rate of protein synthesis measured by the incorporation of [^{3}H]-tyrosine to proteins. The tungstate depended increase in protein synthesis was mediated through the phosphorylation of mTOR and was blocked upon incubation with rapamycin. The activation of mTOR pathway by tungstate was dependent of the phosphorylation of ERK 1/2. In agreement with the involvement of mTOR signaling pathway in the tungstate effects on protein synthesis, tungstate was able to increase phosphorylation of mTOR downstream substrates as S6K1 or 4eIF4. Thus, sodium tungstate could be a useful agent for the treatment of some catabolic situations leading to muscle wasting.

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P06-111
Tumor Necrosis Factor-alpha (TNF-alpha) stimulates the epithelial-mesenchymal transition regulator Snail in cholangiocarcinoma

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Epithelial-mesenchymal transition (EMT) is a series of events during which epithelial cells lose many of their epithelial characteristics and take on properties that are typical of mesenchymal cells that lack of cell-cell adhesion properties. EMT may be activated by various types of growth factors or inflammatory cytokines. In many types of epithelial cancers, the EMT-derived tumor cells are susceptible to metastasis. During tumor progression, epithelial cells acquired a gene-expression pattern closely resembling that mesenchymal cell. This study aimed to investigate the expression of the EMT-associated transcription factor Snail and an adhesion molecule E-cadherin in CCA tissues. Effect of TNF-alpha on EMT activation in CCA cells was also demonstrated. The qRT-PCR analysis revealed that Snail expression is significantly increased in CCA ($p = 0.01$) and correlated with tumor metastasis ($p = 0.02$). The expression of Snail was inversely associated with E-cadherin ($p = 0.004$). The stimulation of TNF-alpha can enhance migration behavior and showed significantly induced the expression of Snail in CCA cell lines whereas expressions of E-cadherin and CK-19 (the epithelial marker) were reduced. Immunofluorescence analysis revealed that TNF-alpha-treated CCA cell lines increased nuclear translocation of Snail

whereas E-cadherin was dramatically decreased. This finding concludes that the changes in the expression of Snail or E-cadherin might regulate the EMT development in CCA resulting in promoting the tumor progression. Overexpression of Snail could provide as a prognostic marker for monitoring the treatment efficiency of CCA patients.

P06-112
Role of FoxO factors in thrombin-mediated proliferation of vascular smooth muscle cells: therapeutic potential for prevention of vein graft failure?

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The most common cause of vein graft failure is intimal hyperplasiawithproliferation of vascular smooth muscle cells (SMC). Thrombin is a well-established inducer of vascular SMC proliferation and migration. Forkhead box-O (FoxO) transcription factors also regulate cell proliferation, apoptosis and cell cycle arrest, but a functional interaction between thrombin and FoxO factors has not been identified to date. In human cultured vascular SMC, thrombin induced a time-dependent phosphorylationand nuclear export of FoxO1 and FoxO3 proteins but not FoxO4. These effectswere mimicked by an activating-peptide (AP) for protease-activated receptor (PAR)-1, and abolished by a PAR-1 antagonist (SCH79797). APs for other PARs were without effect. FoxO1 and FoxO3 phosphorylation were inhibited by the PI3kinaseinhibitor LY294002 while inhibitors of ERK1/2 (PD98059) or p38MAPK (SB203580) were ineffective. Accordingly LY294002 alsoprevented thrombin-stimulated SMC mitogenesis and proliferation. Suppression of FoxO1 and FoxO3 with siRNA augmented basal DNA synthesis and proliferation of SMC. Thrombin also reduces expression of the cell cycle regulating genes $p21^{CIP1}$ and $p27^{kip1}$. LY294002 restored $p21^{CIP1}$ and $p27^{kip1}$ protein expression. Immunohistochemistry revealed that human native and failed saphenous vein grafts were characterized by cytosolic phosphorylated FoxO factors in SMC, in co-localization with $p21^{CIP1}$ and $p27^{kip1}$. In conclusion, thrombin and FoxO factors functionally interact through PI3 kinase/Akt-dependent FoxO phosphorylation leading to expression of cell cycle regulators and ultimately SMC proliferation. This may contribute to remodeling and failure of vein bypass grafts.

P06-113
Crosstalk between NFkappaB- and p53-dependent signaling pathways – functional interference in human colon carcinoma cell line (HCT116)

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Signaling pathways that depend on p53 or NFκB transcription factors are essential components of cellular responses to stress. In general, p53 is involved in induction of apoptosis, while NFκB exerts mostly anti-apoptotic functions. A mathematical model describing functional interactions between this pathways has been recently constructed by Puszynski *et al.* (2009), suggesting different functional output (enhanced survival or apoptosis) depending on the time sequence of each pathway activation. Here we aimed to verify experimentally effects of activation NFκB-dependent pathway on induction of apoptosis and expression levels of p53-dependent genes and proteins.

Colon carcinoma HCT116 cells were incubated with TNFα cytokine to induce NFκB, and/or treated with UV/IR radiation to induce p53 pathway; both factors were used in two different time combinations: TNF stimulation was placed either before or after irradiation. Cells were analyzed at 24 hour time point after irradiation. DNA damage-induced apoptosis was determined by measurement of the sub-G1 cell fraction, expression levels of selelcted p53-dependent genes (*TP53, CDKN1A, NOXA*) and proteins (p53, p21) were assessed by QRT-PCR and Western-blotting. We obserwed that pretreatment with TNFα plays anti-apoptotic, cytoprotective function (reduction in the frequency of apoptotic cells in UV-irradiated p53-proficient cell line and down-regulation of p53 protein). Cell treatment with TNFα after irradiation caused up-regulation of pro-apoptotic *NOXA* gene and down-regulation of p21 protein, suggesting that activation of NFκB which precede p53 activation plays pro-apoptotic, cytotoxic function.

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P06r-114
Molecular basis of the inhibition of store-operated calcium entry by resveratrol

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Resveratrol, a natural phytoalexin found in the skin of red grapes shows a wide range of health-promoting benefits, including inhibition of cancer cells growth, prevention of platelet aggregation, and protection against oxidative stress. Many studies have found diverse targets for resveratrol, including cell cycle regulators, transcription factors, angiogenic and metastatic factors, and regulators of apoptosis/survival pathways. Despite of the importance of the cytosolic free Ca^{2+} concentration in cell signaling, much less is known regarding the effects of resveratrol on the intracellular Ca^{2+} homeostasis. In this study we focused on the molecular basis of the inhibition of store-operated Ca^{2+} entry (SOCE) mediated by resveratrol. We found that resveratrol inhibits SOCE with a $K_{0.5}$ within 5–10 μM range in HEK293 cells stably transfected with tagged-STIM1. STIM1 is a transmembrane endoplasmic reticulum (ER) protein that acts as a Ca^{2+} sensor within this intracellular compartment. Under store depletion conditions, STIM1 multimerizes and translocates to ER-plasma membrane junctions where it activates store-operated Ca^{2+} channels. However, resveratrol inhibits the multimerization of GFP-STIM1, triggered by thapsigargin in HEK293 cells, with a similar kinetics to that found for SOCE inhibition. Also, resveratrol inhibits both ERK1/2 activation and STIM1 phosphorylation at ERK1/2 target sites. Because alanine substitution mutants of STIM1 ($STIM1^{S575A/S608A/S621A}$) show a slower multimerization than wild-type STIM1, and cells expressing these mutants show defective SOCE, we concluded that inhibition of STIM1 phosphorylation by resveratrol contributes to SOCE inhibition.

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P06-115
Secretion of PAI-2 from endothelial cells activated with inflamatory stimuli

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Plasminogen activator inhibitor type 2 (PAI-2) is a widely expressed serpin in various cells and is upregulated in patophysiological conditions like pregnancy, inflammation and infection. Its primary physiological function thought to be the regulation of plasminogen activators in the extravascular compartments. Lately PAI-2 has been proposed to play an intracellular role in tumor metastasis, embryo implantation and macrophage survival. Due to the lack of N-terminal signal peptide, PAI-2 accumulates in the cells and its extracellular concentration is low, but significantly increases during inflammation in response to many proinflammatory stimuli. The exact mechanism by which PAI-2 is secreted has not been yet elucidated.

In this study, we have evaluated the intracellular targeting of PAI-2 by immunoglod staining followed by electron microscopy analysis. We discovered that PAI-2 gathers in vesicular structures at the Golgi and in the endothelial cell periphery. These vesicles stained positive for the *trans*-Golgi network marker TGN46. PAI-2 was delivered to plasma membrane, apparently together with TGN46 in the same vesicles, which after fusion with membranes released cargo. Secretion of PAI-2 was partially inhibited by brefeldin. The secreted PAI-2 was predominantly in its nonglycosylated 47 kDa form as evaluated by Western immunoblotting.

In summary, PAI-2 is distributed between cytosol and nucleus but in endothelial cells activated by LPS enters the *trans*-Golgi network. Our data show that PAI-2, despite the lack of a classical secretory signal sequence, does have the limited potential to enter the ER–Golgi pathway. This Golgi-mediated delivery mechanism of PAI-2 could precede its secretion from endothelial cells activated by inflammatory stimuli.

P06-116
Protein misfolding and oxidative stress compromise heat stress adaptation

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The heat shock response protects proteins from misfolding and aggregation, is a critical regulator of longevity and is a biomarker in aging in *Caenorhabditis elegans*. Aging is characterized by a progressive increase in oxidative stress, an accumulation of cellular damage and denatured proteins, and an impaired stress adaptation. However, the effects of oxidative stress and misfolded proteins on thermotolerance and on the heat shock response remain largely unclear. To address the impact of the general impact of protein misfolding on cell physiology, we introduced two novel primate cell models of destabilized proteins devoid of cellular function, as inert misfolded proteins (IMPs). Characterization of GFP-degron (degron) and a mutant chloramphenicol-acetyltransferase fused to GFP (Δ9CAT) form perinuclear aggregates, are degraded by the proteasome and co-localize with and induce the chaperone Hsp70 in COS-7 cells. We find that IMPs neither significantly compromise chaperone-mediated folding capacity, nor induce cell death. However, they do induce growth arrest and promote stress-induced death upon proteasome inhibition by MG-132 and heat shock. Finally, we show that overexpression of all heat-shock factor-1 (HSF1) and Hsp70 proteins, as well as wild-type and deacetylase-deficient (H363Y) SIRT1, rescue survival upon stress, implying a noncatalytic action of SIRT1 in response to protein misfolding.

To investigate the effect of oxidative stress on responses to heat stress, we exposed mammalian cells to H_2O_2. We report that hydrogen-peroxide (H2O2) pretreatment inhibits both acquired thermotolerance and heat-induced Hsp70 expression in mammalian cells, as well as acquired thermotolerance in the nematode *C. elegans*, via RNA interference. Moreover, we demonstrate that elimination of RNA interference by silencing key enzymes in microRNA biogenesis, *dcr-1* or *pash-1*, restores the diminished intrinsic thermotolerance of aged and H2O2-elimination compromised (catalase-2 and peroxiredoxin-2 deficient) worms.

As a summary, our studies identify novel mechanisms that compromise stress adaptation and may contribute to aging.

P06-117
The metabolic sensor SIRT1 is a novel client of Hsp90

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SIRT1, a mammalian ortholog of Sir2 (silent information regulator 2), is an NAD^+-dependent deacetylase that acts as a metabolic sensor of NAD^+ levels and modulates cellular metabolism and stress resistence. SIRT1 has been implicated in fundamental cellular process, such as cancer and aging, through deacetylation of key signaling molecules, such as p53, E2F, FOXO, PPARγ and PGC-1. Extra copies of the invertebrate Sir2 orthologs have been reported to extend lifespan from yeast to *Drosophila*, while mice deficient in the major mammalian homolog SIRT1 age prematurely. Hsp90 is an evolutionarily conserved molecular chaperone involved in the folding, stabilization and activation of various proteins, so called clients. The growing list of Hsp90 client proteins include kinases (Raf, Akt), transcription factors (p53, steroid receptors), polimerases (telomerase) and many other proteins involved in a wide range of biological processes under both normal and stress conditions. Long term inhibition of Hsp90 cause proteasomal degradation of its clients, therefore Hsp90 is a promising target of anti-cancer drugs. Since SIRT1 has a hydrophobic NAD^+-binding domain, we hypothesized that SIRT1 needs Hsp90 for its function. Here we show that the specific Hsp90 inhibitor geldanamycin (GA) induces degradation of SIRT1 in a concentration- and time dependent manner in mammalian cells. The effect of Hsp90 inhibition is specific to SIRT1 as SIRT2 level is not changed by GA. SIRT1 appears in the detergent insoluble pellet upon combination treatment by GA and the proteasome inhibitor MG132. Moreover, we observe a GA-sensitive physical interaction of SIRT with Hsp90 by immunoprecipitation. Immunofluorescence studies show that GA translocate nuclear SIRT1 to the cytoplasm. We conclude that the metabolic sensor Hsp90 is required for SIRT1 folding and compartmentalization, establishing SIRT1 as a novel Hsp90 client. Inhibition of SIRT1 may contribute to the potent anticancer action of Hsp90 inhibitors. We propose that Hsp90 may modulate metabolic signaling via SIRT1, which may have implications in aging.

P06-118
Molecular mechanisms of co-chaperones CDC37/HARC in the regulation of HSP90 function: potential therapeutic targets in cancer cells

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The HSP90 chaperone protein has a key function regulating many cellular processes that are implicated in cancer, such as proliferation, cell cycle progression, survival, invasion and metastasis. Interest in the possibilities of its therapeutic exploitation has expanded considerably in recent years and has stimulated the development of numerous HSP90 inhibitors, several of which are now in the clinic, including NVP-AUY922 discovered in our Unit in collaboration with Vernalis.

In this project we aim to investigate and characterize the role of the HSP90 co-chaperones CDC37 and HARC in cancer cells, giving special attention to IGF1R-dependent cancer types. We showed that CDC37 and HARC are involved in the regulation of cancer cell proliferation and survival. We demonstrated that IGF1R interacts with CDC37 and that silencing either CDC37 or HAR inhibits significantly the proliferation of rhabdomyosarcoma and colon cancer cells (with a simultaneous increase of apoptosis), sensitizing these cells to treatment with the HSP90 inhibitor 17-AAG. We have also demonstrated that a dual knock-down of both CDC37 and HARC has a greater impact on proliferation and apoptosis in cancer cells, compared to either co-chaperone alone, which indicates a possible redundancy of biological function.

Based on these novel results we hypothesize that a dual inhibition of CDC37 and HARC would be necessary to achieve strong effects in the cellular proliferation, apoptosis and sensitization to treatment of cancer cells. We propose that these two co-chaperones might have similar biological functions, with a certain degree of redundancy, but with different client protein selectivity. However, further studies are required to fully understand these mechanisms.

These novel findings are very exciting as they implicate important roles for CDC37 and HARC as critical co-chaperones involved in chaperoning HSP90 clients. Moreover, deliverables arising from this project should include potential biomarkers for use in patient selection or/and possible new targets for therapeutic intervention.

P06r-119
Kinin B1 and B2 receptor activation reduces collagen I secretion through PKC-PLA2-COX2 participation in rat neonate cardiac myofibroblast

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Neonatal rat cardiac fibroblasts (CF) by the action of TGF-β1 differentiate into cardiac myofibroblasts (CMF), a highly secretory cell phenotype of extracellular matrix proteins and responsible for the healing process after cardiac injury. Bradykinin (BK) activates the B2 receptor (B2R) in CF resulting in the release of nitric oxide and prostaglandins, which ultimately leads to reduced secretion of collagen. However, the presence and role of B2R and kinin B1 receptor (B1R) have not been characterized in CMF. CF were differentiated by TGF-b1 to CMF. The changes induced by B1R and B2R agonists (DAKD and BK, respectively) in collagen I (Col I) secretion were evaluated by inmunowestern blot (WB). We used PKC-PLA2-COX2 inhibitors to evaluate the participation of these proteins in Col I secretion. Also, we stimulated CMF with kinins and tested prostaglandin (PGI2 and PGE2) secretion by enzyme inmuno assay. CMF stimulated with DAKD and BK 100 nM reduced Col I compared with control. PKC inhibition (GÖ6983 100 nM) prevented Col I reduction produced by kinins. PLA2 inhibitor (MAFP 1 μM), COX1-2 inhibitor (indometacin 10 μM) and COX2 specific inhibitor (Rofecoxib 10 μM) also prevented kinins effect. However, COX-1 specific inhibitor (SC560 10 nM), was unable to prevent Col I reduction induced by DAKD and BK. On the other hand, both kinins were capable to increase PGI2 but not PGE2 secretion. Conclusion: The B1R and B2R activation by their agonists decreased Col I in CMF, by PKC-PLA2-COX2- signaling pathways.

P06-120
Role of Notch1 homodimerization in human T-cell development and T-ALL

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Despite using a rather simple pathway, Notch signaling exerts a multitude of very diverse cellular effects. A prominent function of Notch lies in promoting and regulating multiple steps of T-cell development. Deregulated Notch signaling has been implicated in tumorigenesis and mutational activation of Notch1 has been shown to be the major oncogenic event in T-cell acute lymphoblastic leukemia and lymphoma (T-ALL). As such, Notch represents an attractive therapeutic target for the treatment of this type of cancer. Unfortunately, however, its usefulness for this purpose is limited by its broad range of physiological functions. Our ability to exploit Notch for the treatment of cancer would be enhanced if oncogenic functions of Notch could be more specifically targeted without interfering with all functions of this pathway.

Recent biochemical studies have shown that different functions of Notch can be separated biochemically. Most target genes of Notch can be transactivated by a single complex of Notch together with the DNA binding factor CSL and the MAML transactivator. In contrast, activation of a specific subset of target genes requires head to head dimerization of two such complexes on their promoters. This dimerization of Notch has been demonstrated to be crucial for both T-cell development and leukemogenesis in mice, making dimeric Notch signaling a potential target for innovative therapeutic approaches. Whether the same applies to human T cell development and T-ALL has not been examined yet.

Therefore, we here investigated whether Notch1 homodimerization is required for differentiation of human hematopoietic stem cells derived from postnatal thymus, employing the OP9 coculture system. Furthermore, we address the need for Notch1 dimerization in mediating the oncogenic functions of Notch1, both in established human T-ALL lines and an *in vitro* model for human T-cell leukemogenesis.

P06r-121
PDGFR inhibitor – tyrphostin AG1296 in regulation of growth and survival of rhabdomyosarcoma (RMS) cells

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An important early event in the development of the neoplastic phenotype is the induction of genes involved in autocrine growth regulation, such as growth factors and their receptors. Platelet-derived growth factor (PDGF) and its receptors are involved in a variety of diseases. In all cases enhanced signaling of the receptor is the hallmark. Therefore, the family of PDGFs receptors emerges as an attractive target in anti-cancer therapy.

In the present work, we tested selective PDGFR inhibitor, tyrphostin AG1296 on rhabdomyosarcoma (RMS) cells. We evaluated influence of investigated compound on proliferation, viability and protein expression.

MTT and crystal violet staining (CV) methods showed that tyrphostins significantly inhibit proliferation of investigated cell line. The AG1296 inhibitor affects RMS cell proliferation in a dose-dependent way at the concentration range 1–100 μM. At concentrations above 25 μM there was 100% inhibition of growth of these cells and a cytotoxic effect was noticed. 50% inhibition of RMS cells proliferation (IC_{50}) was observed at concentration 7.76 ± 0.35 μM. Differential staining with Hoechst 33258/PI showed that PDGFR inhibitor induce apoptosis of rhabdomyosarcoma cell. Western Blot technique showed that tested inhibitor could influence on expression of main signal proteins.

The results of these experiments indicate that autocrine growth of RMS cells is regulated by at least one autocrine loop, involving platelet-derived growth factor (PDGF) and its receptor (PDGFR). The fact that tyrphostin AG1296 is able to complete inhibition of RMS cell growth *in vitro* gives a chance for providing a new group of antitumor drugs, which may be more effective than the medicines used so far.

P06-122
Lipid signaling in the nucleus: potential regulation of Ebp-1 mediated transcriptional repression by phosphoinositides

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ErbB3 binding protein 1 (Ebp1) is a conserved protein involved in proliferation, differentiation and apoptosis. In particular, Ebp1 inhibits cell growth in breast and prostate cancer cells. Epb1 interacts with the retinoblastoma protein and the resulting complex play a role in transcriptional inhibition of genes involved in cell cycle regulation, such as the Cyclin-D1 gene. In a previous study, we identified Ebp1 as a potential nuclear phosphoinositide-binding protein by lipid pull down and quantitative mass spectrometry[1]. Further studies to validate the interaction showed that Ebp1 demonstrated *in vitro* binding activity to four phosphoinositides PtdIns3*P*, PtdIns4*P*, PtdIns5*P* and PtdIns(3,5)P_2. In addition, generation of deletion constructs revealed that Ebp1 may have two or more PI-binding sites consisting of patches of basic residues. Finally, we explored the possible functional role of Ebp1-phosphoinositide interaction. Using luciferase reporter assays, we show that the overexpression of the nuclear PI3 kinase class 2α, which synthesizes PtdIns3*P* from phosphatidylinositol, enhanced Ebp1-mediated transcriptional repression of the cyclin D1 gene. Taken together, these results suggest that Ebp1 may serve as a phosphoinositide effector protein. In particular, the PtdIns3*P*-binding properties of Ebp1 may have a role in transcriptional regulation of the Cyclin-D1 gene.

Reference

[1] AE Lewis *et al.* (2011) Identification of nuclear phosphatidylinositol 4,5 bisphosphate-interacting proteins by neomycin extraction. Mol Cell Prot 10(2): 10, 1074.

P06r-123
Inhibiotion of CXCR4 and CXCR7 receptor activity as a novel strategy for targeting cervical cancer

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Cervical cancer (CC) is one of the most common cancer in women in developing countries. Usually, the majority of tumors are diagnosed at advanced stages what results high mortality. CC cells demonstrate the expression of G-protein coupled seven transmembrane domain receptors name as chemokine receptors such as CXCR4 and CXCR7 which play an important role in cancer development. The aim of this study was to investigate the role of CXCR4 and CXCR7 receptor in biology of CC cell line (HTB-35).

HTB-35 cell line with stable down-regulation of CXCR4 and CXCR7 gene was prepered using lentiviral RNAi – expression system. To verify gene knockout real-time RT-PCR, western blot and FACS analysis was performed. To asses proliferation MTT assay and cell counting in hemocytometer was done. Cell cycle analysis and apoptosis was studied by flow cytometry. The expression level of genes related with angiogenesis and metastasis was performed by real-time RT-PCR. Chemotaxis was estimated using modyfied Boyden chamber. Colony forming assay was used to indentify the ability of propagation *in vitro*. Mouse model was used to examinate the influence of CXCR4 and CXCR7 receptor on tumour growth.

We observed that simultaneously downregulation of CXCR4 and CXCR7 gene activity resulted proliferation decrease. We observed modutation level of transcription factors, metalloproteinases and their inhibitors. Results suggest that CXCR4 and CXCR7 receptors play an important role in many biological pathways of CC cell line and cells with stem cell properties. We suppose that both, CXCR4 and CXCR7 receptors are one of the most important target in tumour therapy.

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P06-124
The social network: testing the communication between tumor cells and the surrounding stroma in tumor development

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It is now generally accepted that a tumor is a heterogeneous entity composed of a wide range of cell populations in different stages of differentiation. Under this assumption, a specific population of cells with stem-like properties have been searched for and successfully isolated from tumors of different origins. These cancer stem cells (CSCs) were later implicated in tumor aggressiveness and resistance to conventional therapies as well as tumor relapse. Aiming to study the molecular mechanisms underlying hexavalent chromium [Cr(VI)] induced lung cancers, we malignantly transformed the normal human bronchial epithelial cell line BEAS-2B into the RenG2 system, using low density culture in the presence of Cr(VI). Two additional cell lines (DRenG2 and DDRenG2) were attained following serial rounds of injection in nude mice. Characterization results allowed us to identify different cellular sub-populations within each cell line, and prompted the hypothesis that CSCs may have driven BEAS-2B' malignization. Sphere-formation assay was used to search and isolate these cells, which were only present in DRenG2 and DDRenG2 cell lines, forming more and bigger spheres in the DDRenG2. This suggested that a dedifferentiation process featured the formation of CSCS during RenG2 derivation in nude mice. To access the involvement of mice stroma in this process, chirurgical-isolated mouse stromal cells of the subcutaneous compartment were co-cultured with RenG2 cells for 30–60 days (time needed to induce tumors in mice with RenG2), which resulted in the emergence of a CSCs sub-population. We are now able to attest that CSCs may emerge in a tumor as a consequence of stromal-emitted paracrine signals, which brings deep implications to future therapeutic approaches.

P06-125
The study of the ligand-dependent signal transduction pathway and its molecular mechanism of GPR120

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Free fatty acid receptors (FFARs) are a member of G protein-coupled receptor (GPCR) family. Among the FFARs, GPR120 whose endogenous ligands are long-chain fatty acids are thought to play important physiological roles in GLP-1 secretion, obesity and inflammatory effects.

Although GPR120 has received increasing attention as attractive drug targets for type 2 diabetes and metabolic diseases, much still remains unclear about pharmacological properties of GPR120 ligand and molecular mechanism of ligand recognition by GPR120. We showed that troglitazone known to as peroxisome proliferators-activated receptor γ (PPARγ) agonist promoted ERK but not $[Ca^{2+}]_i$ response in HEK293 cells expressing GPR120. Also, the time-course analysis of ERK activation revealed that the transient ERK response induced with the endogenous ligand α-LA and synthetic agonist NCG21, were partly mediated through $G_{\beta\gamma}$-PLC pathway. On the other hand, troglitazone-induced sustained ERK response was partly mediated by β-arrestin pathway. Further, mutagenesis study of GPR120 revealed that Arg99 residue contributed to both ERK and $[Ca^{2+}]_i$ activation induced with these ligands and that some residues located in the binding cavity might contribute to the sustained ERK response induced with the troglitazone. Taken together, the pharmacological properties of these ligands and its molecular mechanism might useful to investigate the further study of biological function of GPR120.

P06-126
Increased expression of Epac2 during *in vitro* tube formation of human microvascular endothelial cells

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Angiogenesis, the process by which new vascular networks are formed from preexisting capillaries, is essential for embryogenesis, development and wound repair, and plays an important role in the progression of diseases such as cancers and diabetic retinopathy. However, the genes regulating changes in cell shape and cell-cell communication necessary for vascular tubulogenesis are still poorly understood. We compared the gene expression between monolayer-cultured and capillary-formed primary human microvascular endothelial cells (HMVECs) by DNA microarray analysis. Then we have found that the expression of Epac2 (exchange proteins activated by cAMP, a guanine nucleotide exchange factor for Rap1) was dramatically increased in Matrigel-driven *in vitro* tubulogenesis. Epac1, another member of Epac family, was also expressed in vascular endothelial cells (ECs), but its fold of change in Matrigel-driven tubulogenesis was smaller than that of Epac2. Addition of VEGF, FGF2 or Forskolin gave only slight increases of Epac 1 and 2 mRNA levels in the monolayer EC cultures. Addition of VEGF or Forskolin slightly increased Epac 2 mRNA level and addition of FGF2 decreased Epac2 mRNA level in the capillary-forming HMVECs. These results suggested that the increased expression of Epac2 during tube formation was mainly related to a capillary-like structure formation of endothelial cells. Preliminary knock-down experiments showed that transfection of an siRNA to Epac2 modestly inhibited the *in vitro* tube formation of HMVECs, suggesting that Epac2 may be involved in the capillary structure formation of ECs. Although more studies are needed to clarify the functions of Epac2 in vascular tubulogenesis, the present findings may provide new clues for understanding the regulatory mechanisms of angiogenesis and vasculogenesis.

P06-127
Chlorogenic acid from the Japanese herbal drug Kinginka (Flos Lonicerae japonicae) confers anti-inflammatory effects

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Rationale: Flowers and buds of the Japanese honeysuckle *Lonicera japonica* Thunberg, known as *Kinginka* (Flos Lonicerae japonicae, FLJ), is included in Japanese Kampo medicine to treat infectious and inflammatory diseases and is popularly used as a Chinese traditional medicine, such as *Yin Hua* tea. It was unknown which constituent of FLJ is responsible for its pharmacological effects.
Methods: FLJ was extracted with methanol and fractionated by hydrophobicity. We measured the effects of each fraction on the induction of the inflammatory mediator nitric oxide (NO), which was induced by IL-1β in primary cultured rat hepatocytes. The expression of inducible nitric oxide synthase (iNOS) was analyzed by Western blot analysis and RT-PCR.
Results: We fractionated the FLJ extract into hydrophobic, butanol-soluble, and water-soluble fractions. Hydrophobic and water-soluble fractions suppressed the induction of NO and decreased the level of iNOS protein in the IL-1β-stimulated cells. Chlorogenic acid, a major constituent of the water-soluble fraction, markedly reduced the levels of NO production, iNOS protein, and iNOS mRNA. Chlorogenic acid also decreased the levels of mRNA encoding inflammatory cytokines and chemokines, similarly to functional foods, flavanol-rich lychee fruit extract (Oligonol) and active hexose correlated compound (Amino Up Chemical Co., Ltd., Sapporo, Japan).
Conclusions: Chlorogenic acid, a major constituent in water-soluble fraction, is mainly involved in the anti-inflammatory effect of the FLJ extract. Our data suggest a therapeutic potential of the FLJ extract and chlorogenic acid for inflammatory diseases.

P06-128
Role of PKCalpha-mediated cofilin phosphorylation in the degranulation from RBL-2H3 mast cells

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Protein kinase C (PKC) activity is essential to the IgE-induced the release of histamine from mast cells. However, the role of individual PKC isoform is controversial and downstream molecules of PKCs are unknown in the degranulation. Using specific inhibitors, kinase-negative mutants and siRNA against PKCalpha or PKCbeta I, we found that PKCbeta I positively regulates degranulation in RBL-2H3 cells, whereas PKCalpha negatively regulates degranulation. Mass spectrometric and mutagenic analyses revealed that PKCalpha phosphorylates cofilin at Ser23 and/or Ser24 during degranulation. Overexpression of a non-phosphorylatable form (S23,24A), but not a mutant mimicking phosphorylated form (S23,24E), increased degranulation. Furthermore, the S23,24A mutant bound to F-actin and retained its depolymerizing and/or cleavage activity; conversely the S23,24E mutant was unable to sever actin filaments, resulting in the F-actin polymerization. In addition, the S23,24E mutant preferentially bound to the 14-3-3zeta protein. FACS analysis with FITC-phalloidin, and simultaneous observation of degranulation, PKC translocation and actin polymerization revealed that during degranulation, actin polymerization was dependent on PKCalpha activity. These results indicate that a novel PKCalpha-mediated phosphorylation event regulates cofilin by inhibiting its ability to depolymerize F-actin and bind to 14-3-3zeta, thereby promoting F-actin polymerization, which is necessary for cessation of degranulation.

P06m-129
Effects of vascular endothelial growth factor (VEGF) on CAIX expression in Hep3B cells

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Zinc containing carbonic anhydrase enzymes have important roles in different physiological processes as gas exchange, ion transport and acid-base balance. Carbonic anhydrases catalyse the reversible reaction from H_2O and $CO_2$to HCO_3^- ions. CA IX is a tumor associated transmembran member of the CA family. CAIX expression very important roles such as malignant cell invasion, adhesion and the regulation of cell proliferation. Carbonic anhydrases IX which is transcriptional regulated by hypoxia related factor, HIF1α, are a crucial enzyme in most cancer types. Cytokines are biological molecules which have roles in regulation of cellular reactions There is rather limited study on the action of any cytokine to expression of CA9 gene. Vascular Endothelial Growth Factor (VEGF) is a crucial mediator for angiogenesis and is also regulated by hypoxic conditions mainly by HIF1-α. The aim of this study was to investigate the CA IX mRNA expression levels in hepatoma cells (Hep3B) treated with VEGF. Therefore, Hep3B cells were treated by VEGF for different time intervals, namely 1, 3, 6, 24, 48 and 72 hour. VEGF upregulates CAIX mRNA expression mainly at 3 and 6 hour. Different concentration of VEGF on CAIX cells were also investigated and optimum dose for maximum expression was determined.
Keywords: Carbonic anhydrase IX, cancer, VEGF, Hep3B

P06-130
A single mutation in Securin induces chromosomal instability and enhances cell invasion

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Pituitary tumor transforming gene (*pttg1*) encodes Securin, a protein involved in the inhibition of sister chromatid separation binding to Separase until the onset of anaphase. Separase is a cysteine-protease that degrades cohesin to segregate the sister chromatids to opposite poles of the cell. The amount of Securin is strongly regulated because it should allow Separase activation when is degraded by the anaphase promoting complex/cyclosome, should arrest the cell cycle after DNA damage, when is degraded through SKP1-CUL1-βTrCP ubiquitin ligase, and its

overexpression induces tumor formation and correlates with metastasis in multiple tumors. Securin is a phosphoprotein that contains 32 potentially phosphorylatable residues. We mutated and analyzed most of them, and found a single mutant, hSecT60A, that showed enhanced oncogenic properties. Our fluorescence activated cell sorting analysis, fluorescence *in situ* hybridization assays, tumor cell migration and invasion experiments and gene expression by microarrays analysis clearly involved hSecT60A in chromosomal instability and cell invasion. These results show, for the first time, that a single mutation in *pttg1* is sufficient to trigger the oncogenic properties of Securin. The finding of this point mutation in patients might be used as an effective strategy for early detection of cancer.

P06-131
Characterization of DNA topoisomerase II alpha as a nuclear phosphoinositide-effector protein

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In the past few decades, the existence of nuclear phosphoinositide (PI) signaling has been well established. A growing body of evidence shows that nuclear PIs regulate important nuclear processes such as gene expression and chromatin remodeling. However, the precise mechanisms by which PIs regulate these processes is unknown. In order to understand PI-regulated nuclear functions, we recently identified and validated DNA topoisomerase II alpha (Topo 2A) as a nuclear PI-binding protein among others. Moreover, we suggested that the binding between Topo 2A and PIs might occur via electrostatic interactions between the negatively charged phosphate groups on PIs and the 7 basic arginine and lysine (K/R) motifs (consensus: K/R-X (n = 3–7) –K-K/R-K/R) concentrated in the C-terminal domain (CTD) of Topo 2A. Here, we further characterized the interaction between Topo 2A and PIs by *in vitro* and *in vivo* studies. Different deletion constructs of Topo 2A-CTD lacking specific K/R motifs were assessed for their binding to various PIs in lipid overlay assays. In general, deleting the K/R motifs but not that of a region devoid of these motifs, led to a decrease in the binding of mutant CTDs to PIs. However, the specific deletion of motifs 4, 5 and 7 resulted in a significant reduction in PI-binding suggesting that more than one K/R motif may be involved in binding to PIs. Furthermore, individual peptides corresponding to the 7 K/R motifs, respectively, were found to be able to relieve PtdIns(4,5)P2-mediated inhibition of Topo 2A decatenation activity in competition studies. Taken together, these results validate K/R motifs in the CTD of Topo 2A as PI-binding sites. On the other hand, immunostaining analyses showed that endogenous Topo 2A colocalized partly with PtdIns(4, 5)*P*2 in nuclear speckles of HeLa cells in log, G0/G1 and S- but not M-phases of the cell cycle. Overall, these results demonstrate the importance of K/R motifs in mediating Topo 2A-PI interactions and show that Topo 2A and PtdIns(4,5)P2 can localize to the same subnuclear structures, suggesting a potential mode of regulation of enzyme activity.

P06-132
SCF-FBW7α targets polo-like kinase 1 for degradation in a cell cycle-dependent manner

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FBW7 is a member of the F-box family of proteins, which function as the substrate-targeting subunits of SCF (SKP1/CUL1/F-box protein) ubiquitin ligase complexes. FBW7 has a central role in cell cycle progression, cell growth and cell differentiation by targeting oncogenic proteins for degradation. In addition, several cancer-associated mutations have been shown to exist in *FBW7*. FBW7 possesses three isoforms (FBW7α, FBW7β and FBW7γ) and has been shown to not only bind the SCF but to also dimerize with itself. Using immunoprecipitation experiments and tandem mass spectrometry, we identified PLK1, a highly conserved serine/threonine kinase that plays critical roles in many cell cycle events, as an FBW7α-interacting protein. PLK1 plays a key role to ensure the normal mitosis. Interestingly, both overexpression and down-regulation of PLK1 are associated with tumour development or with aneuploidy induction and tumour formation *in vivo*, respectively. Therefore, a normal level of PLK1 is important for mitosis. In this study, we found a physical interaction between tagged-FBW7α and PLK1 by reciprocal coimmunoprecipitation from both nuclear and total extracts of cells treated with a proteasome inhibitor. To determine if endogenous FBW7α regulates the degradation of endogenous PLK1, we used knockdown experiments and we detected an increased amount of PLK1 in U2OS cells interfered with siRNA-*FBW7* and a longer half-life of the protein. Moreover, we observed that this accumulation occurs in S and G2 phases of the cell cycle. In addition, ubiquitination assays showed that PLK1 degradation is mediated by the proteasomal degradation pathway. These results and its implications for cell cycle regulation will be discussed.

P06-133
Downregulation of protein tyrosine phosphatase PTPL1 alters cell cycle and upregulates invasion-related genes in prostate cancer cells

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Protein tyrosine phosphatase L1 (PTPL1, also known as PTPN13, FAP-1, PTP-BAS or hPTP1E), a non-receptor type tyrosine phosphatase, has been involved in the regulation of apoptosis and invasiveness of various tumour cell types. We have recently reported (1) that downregulation of PTPL1 by small interfering RNA in PC3 prostate cancer cells decreases cell proliferation and concomitantly reduces the expression of cell cycle-related proteins such as cyclins E and B1, PCNA, PTTG1 and phospho-histone H3. PTPL1 downregulation also increases the invasion ability of PC3 cells through Matrigel coated membranes. cDNA array of PTPL1-silenced PC3 cells versus control cells showed an upregulation of invasion-related genes such as *uPA*, *uPAR*, *tPA*, *PAI-1*, *integrin α6* and *osteopontin*. This increased

expression was also confirmed in PTPL1-silenced DU145 prostate cancer cells by quantitative real time PCR and Western blot. Correlations of the expression of these proteins in tissues from clinical biopsies are proposed. These findings suggest that PTPL1 is an important mediator of central cellular processes such as proliferation and invasion.

Reference

[1] Castilla C, *et al*. Clin Exp Metastasis 2012; 29:349–358.

P06-134
Ubiquitin-mediated lysosomal degradation of cyclin-dependent kinase 1

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In mammalian cells, Cyclin-dependent kinase 1 (Cdk1) has been primarily implicated in G2/M transition, mainly in association with cyclin B. Given its essential role in cell cycle, Cdk1 is highly regulated by its cyclin partner and by phosphorylation and dephosphorylation and changes in subcellular localisation. However, little is known about the proteolytic control of Cdk1. Recently, it has been shown that Cdk1 was downregulated under genotoxic stresses, that double-stranded RNA-activated protein kinase (PKR) was involved in the process and that PKR-mediated Tyr4-phosphorylation facilitates Cdk1 ubiquitination and proteosomal degradation. Nevertheless, the Cdk1-specific E3 ubiquitin ligase remains to be identified. Here we show that βTrCP, a substrate-interacting subunit of Skp1/Cul1/F-box protein ubiquitin ligases, coimmunoprecipitated with Cdk1. Moreover, overexpression of HAβTrCP induced a marked reduction of Cdk1, and downregulation of βTrCP caused an accumulation of Cdk1. In addition, the half-life of Cdk1 increased in βTrCP siRNA interfered cells compared with GFP siRNA cells. Furthermore, ubiquitination experiments showed that $SCF^{\beta TrCP}$ ubiquitinates Cdk1 both *in vivo* and *in vitro*. However, when we analysed the effect of doxorubicin treatment or the PKR activation on Cdk1, we found an increase in the amount of this protein. This effect was reversed by PKR downregulation using siRNA, and was not observed in βTrCP siRNA interfered cells. Interestingly, we detected an accumulation of Cdk1 in cells treated with a lysosomal inhibitor but not a proteasomal inhibitor. We propose a mechanism for the proteolytic control of Cdk1 involving its ubiquitination by $SCF^{\beta TrCP}$ and its degradation via lysosome. Genotoxic stress triggered by PKR-mediated phosphorylation could prevent this degradation.

P06-135
The response to HB-EGF in human renal cell lines is impaired by downregulation of protein kinase CK2

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Heparin binding EGF-like growth factor (HB-EGF) has been shown to bind and stimulate EGFR and ErbB4, triggering the activation of different signaling pathways, some of which are also regulated by the protein kinase CK2. The involvement of CK2 in the response to HB-EGF has been studied using CK2 pharmacological inhibitors (TBB and CX-4945) and CK2α- or CK2β-silenced (ΔCK2α and ΔCK2β, respectively) HK-2 (human tubular proximal cells) and 786-O (human ccRCC) cell lines. Downregulation of CK2α had negative effects on ERK1/2 activation (P-Thr202, P-Tyr204) in response to HB-EGF in either HK-2 or 786-O cells. Moreover, HB-EGF stimulation of PKB (P-Ser473) was less sustained in ΔCK2αHK-2 cells than in control HK-2. 786-O cells are PTEN negative and show constitutive activation of PKB. In this cell line, HB-EGF promoted only a slight increase in P-PKB(Ser473) which was little affected by downregulation of CK2α. On the other hand none of the effects of HB-EGF on these signaling pathways were significantly affected by depletion of CK2β in either HK-2 or 786-O cells. This indicates that free CK2α can replace CK2 holoenzyme in sustaining the response to HB-EGF. Moreover, the differences in PKB activation detected in HK-2 and 786-O cells suggest that the CK2 effects on this pathway requires PTEN. In agreement with this, TBB and CX-4945 inhibited PKB activation in response to HB-EGF in HK-2 cells but had little effect in 786-O cells. All these data support the involvement of CK2 in the cell response to HB-EGF. Supported by grants BFU2009-10189 (MCINN) and FIS PI081351 (Instituto de Salud Carlos III).

P06-136
Downregulation of the catalytic and the regulatory subunits of protein kinase CK2 has different consequences on cell proliferation and phenotype changes in HK-2 and 786-O cells

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Protein kinase CK2 has a tetrameric structure, composed of two catalytic (CK2α or CK2α') and two regulatory (CK2β) subunits. Alterations in CK2 activity and/or in the ratio of its subunits occur in various pathological processes including clear cell renal cell carcinoma (ccRCC). To study the functional consequences of the unbalance between CK2 subunits we used the HK-2 (human tubular proximal cells) and 786-O cells (human ccRCC) to generated CK2α- or CK2β-silenced (ΔCK2α and ΔCK2β) cell lines by lentiviral transfection. In 786-O and HK-2 cells, ΔCK2α also caused a decrease in CK2β (suggesting a decrease in CK2 holoenzyme), but no decrease in CK2α was detected in ΔCK2β cells (suggesting an increase in free CK2α levels). ΔCK2β caused a decrease in cell proliferation in both 786-O and HK-2 cells whereas the effects of ΔCK2α depended on the cell type. ΔCK2α HK-2 cells proliferate much faster than control cells whereas ΔCK2α had minor effects on 786-O cell proliferation. On the other hand, ΔCK2β 786-O cells migrated faster than ΔCK2α and control 786-O cells in wound healing assays. ΔCK2α did not significantly alter the epithelial morphology of either 786-O or HK-2 cells. In contrast, ΔCK2β 786-O and HK-2 cell lines showed an elongated fibroblast-like phenotype, suggesting an epithelial to mesenchymal transition. In agreement with this, ΔCK2β cells showed decreased ratios in E-cadherin/N-cadherin and in Slug levels respect to control 786-O and HK-2 cells. Interestingly, E-cadherin and Slug levels also decreased in ΔCK2α cells. However, ΔCK2α also caused a decrease in N-cadherin levels in both cell lines what could help to explain the differences in phenotype

caused by ΔCK2α and ΔCK2β. Supported by grants BFU2009-10189 (MCINN) and FIS PI081351 (ISCIII).

P06-137
Overexpression of Dyrk1a in neural progenitor cell cultures avoids the antiproliferative effect of Hcy

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Previous results from our lab show that hyperhomocysteinemia (HHcy), abnormally elevated plasma levels of homocystein (Hcy), decreases neurogenesis within the adult brain, concomitant with a decrease on the tissue methylation potential. Using subventricular zone (SVZ)-derived neural precursor cell cultures, we have shown that Hcy interferes specifically with bFGF signaling pathways, decreasing the bFGF-induced Erk1/2 phosphorylation and cyclin E expression, leading to a decrease on proliferation, without altering other similar growth factor signaling like that of EGF. Hcy induced the expression of Sprouty2 (Spry2), a dual regulator which has a methylation-sensitive promotor and is able to inhibit bFGF signaling when dephosphorylated. In short-term treatments, Hcy induced the accumulation of the non-phosphorylated form of Spry2, and also increased the expression of Spry2-phosphatase PP2A and decreased the expression of one of Spry2 kinases, Dyrk1A, which has been implicated in the regulation of SVZ neurogenesis. To test whether higher levels of Dyrk1A may overcome the antiproliferative effect of Hcy, we overexpressed the Dyrk1A gen in neural precursor cells, in the absence or presence of Hcy. Increased levels of Dyrk1A promoted the phosphorylation of Spry2, driving it to the proteasome. Dyrk1A also increased the number of proliferating (Ki67+) cells, both in the absence and presence of Hcy. In conclusion, Dyrk1A controls bFGF signaling in neural progenitor cells by phosphorylating and sending to proteasomal degradation the bFGF negative regulator Spry2.

P06-138
Evidence of human sialidase Neu3 involvement in colorectal carcinogenesis

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Human sialidase NEU3, an enzyme of the glycosidase family, catalyzes the hydrolytic cleavage of nonreducing terminal sialic acid residues linked to glycoconjugates oligosaccharidic chains, regulating the lipid bilayer sialic acid content and the modulation of gangliosides content. It was shown that HsNEU3 is involved in colorectal cancer (CRC). NEU3 seemed to inhibit apoptosis, but also to activate the epidermal growth factor receptor (EGFR) pathway; in literature, a correlation was established in HeLa cells between NEU3 overexpression and EGFR phosphorylated protein expression, whereas silencing resulted in the opposite. EGFR activation affected KRAS and MAP kinase pathway, resulting in cell differentiation and proliferation. In collaboration with Istituto Cantonale di Patologia (Locarno), tumor tissues from CRC patients surgically resected were analyzed by Real-time PCR and a frequent correlation was observed between NEU3 and EGFR mRNA levels. These data were confirmed on human colon cancer cell lines. We studied the effect of NEU3 transfection of different human colorectal cancer cell lines with pcDNA3. X-HsNEU3-HA construct. Protein expression was investigated by Western blot analysis. Cells transfected showed increased EGFR and MAP kinase (MEK 1/2 and ERK 1/2) phosphorylation and Akt activation. In addition, it was observed that NEU3 overexpression causes lipid phosphatase PTEN reduction that inhibits Akt, but not MAP kinase pathway. Since NEU3 is involved in EGFR pathway, it might be considered a good molecular target for CRC patients resistant to Panitumumab and Cetuximab, drugs that target epidermal receptor and cause suppression of its activation.

P06-139
Pro-inflammatory SAPK pathways are inhibited by ligand activated LXR

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Increasing amount of evidence states a close link between metabolism and immunity. Certain nuclear receptor pathways (particularly PPARs and LXRs) and stress activated protein kinase (SAPK) pathway are both shown to be important regulators of glucose and lipid metabolism as well as immune response. Since they have opposite effects on these processes, the existence of a biologically relevant negative cross-talk between these signaling pathways is very likely. As an example of this interaction, we have previously shown that thiazolidinediones, which are synthetic PPARγ ligands used as insulin-sensitizing agents in medicine for treatment of type 2 diabetes, mediate their hypoglycemic action by inhibition of the JNK cascade. In addition to PPARγ, we have extended our research to other members of the NR family, analyzing their ability to inhibit the activation of the JNK and p38 pathway. Our data led us to focus on LXRs which has the strongest inhibitory effect. We showed that the effect of the ligand is receptor specific by studies on LXR double knockout mice. And also our results indicate that MKP1, a phosphatase that is known to inhibit JNK activity, is not responsible of this effect. According to our data, the inhibition by these NRs seems to be mediated by a transcriptionally-dependent mechanism. Therefore gene products mediating such interference will be potentially relevant in biomedicine, particularly in the context of chronic inflammatory diseases such as insulin resistance and type 2 diabetes or atherosclerosis.

P06-140
Receptor-like protein tyrosine phosphatase μ dephosphorylates and interacts with phospholipase Cγ2

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RPTPμ is a type IIB receptor protein tyrosine phosphatase that mediate homophilic cell-cell interactions via its ectodomain whereas it can modulates the phosphorylation state of cadherin/catenin complexes via its intracellular catalytic domain. RPTPμ has been localized in tissues where tight intercellular contacts are physiologically important, such as the vascular endothelia, lung epithelia and the intercalated discs of cardiomyocytes. Although, a series of interactors and substrates of RPTPμ have been so far identified, however the precise function of RPTPμ at the cellular level is still poorly understood.

To gain insight into the signaling pathway downstream RPTPμ, we focused on identifying new potential physiological

substrates of this receptor PTP. Our previous work brought evidence that RPTPμ preferentially dephosphorylated a phosphopeptide identical in sequence to that surrounding tyrosine Y753 of human phospholipase Cγ2 (PLCγ2). Data from the literature revealed that PLCγ2 plays a central role in signal transduction via Ca^{2+}. Also, its catalytic activity is essentially dependent on the phosphorylation of two tyrosines (Y753 and Y759) situated into the SH2-SH3 liker region of PLCγ2. In the present approach, we investigated if RPTPμ is able to modulate the phosphorylation state of the two essential tyrosine residues of PLCγ2. By *in vitro* experiments, we demonstrated that the catalytic domain of RPTPμ was able to dephosphorylate both phosphotyrosines of PLCγ2. Moreover, we showed using immunoprecipitation that RPTPμ directly interacts with PLCγ2, when they are endogenously or overexpressed in mammalian cells. Our results suggest that PLCγ2 is a native substrate of RPTPμ, possibly being an important component of the signal transduction pathway(s)-mediated by RPTPμ.

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P06-141
Cross-talk between signalling pathways and sphingolipid metabolism: looking for new players in Sphingolipid homeostasis

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Sphingolipids play key cellular roles as structural components of membrane bilayers and as signalling molecules that participate in important physiological and pathological processes. While first regulatory mechanisms of sphingolipid metabolism start to emerge we do not have a good understanding of the overall control of this route. Very recent results obtained by the Riezman lab show that deletion mutants of kinases and phosphatases implicated in nutrient signalling pathways have an interesting differential sphingolipid profile. We will use this powerful tool to find new regulators of sphingolipid homeostasis. The lipidomics of signalling pathway mutants shows two groups of mutants that specifically accumulate ceramides or complex sphingolipids. To perform a functional analysis of these mutants I will use *S. cerevisiae* as cell-model system combining genetic analysis, lipidomics studies, and *in vitro* and *in vivo* assays.

P06-142
Arhgap12 as a novel binding partner of GULP regulates Rac1 activity during Stablin-2-mediated cell corpse engulfment

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Stabilin-2 is a phosphatidylserine receptor that mediates the clearance of apoptotic cells. An adaptor protein, GULP, directly interacts with stabilin-2 viaits PTB domain and plays a key role in stabilin-2-mediated phagocytosis. However, mechanisms that control the stabilin-2-mediated cell corpse engulfment through actin rearrangement are largely unknown. Herein, we identified ArhGAP12 as a novel partner of GULP. ArhGAP12 interacted with the proline-rich motif of GULP via its Src homology 3 (SH3) domain and dissociated from GULP upon stimulation of stabilin-2. Knockdown of ArhGAP12 increasedstabilin-2-mediated phagocytosis, while overexpressionof ArhGAP12 decreased phagocytosis. ArhGAP12 specifically controlled the activity of Rac1 but of not RhoA. Therefore, inhibition of Rac1 activity by ArhGAP12 led to the decrese of stabilin-2-mediated phagocytosis. Taken together, these results indicate that ArhGAP12 is a novel partner of GULP and plays an important role in stablin-2-mediated cell corpse engulfment by the regulation of rac1 activity.

P06-143
Characterization of p53 activator compounds which inhibit a survival factor kinase as well

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p53 is a tumor supressor protein which plays a crucial role in cell cycle regulation and apoptosis induction thus functions as a tumor supressor that is involved in preventing cancer development.

In the literature several compounds were described as p53 activators, which regenerate the active conformation of P53 or induce the expression of the protein. The aim of our study was to identify new small molecular activators.

For the first step we synthetized the analogues of well known p53 activators and we tested these compounds in cell viability assay on 18 different cell lines [included p53 expressing and p53 knock out (−/−) cells]. We found that one of the reference compound and it's analogues also eradicated p53 (−/−) cells. Therefor we further investigated the effect of these compounds searching for other optional target.

As a result we identified a kinase survival factor (FLT3) which is a potential target of these compounds making them multiple targeted inhibitors.

P06-144
Regulation of cell cycle in response to osmostress

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Exposure of yeast to osmostress results in rapid activation of the stress-activated protein kinase (SAPK) Hog1. In response to stress, Hog1 modulates several aspects of cell biology, such as changes in gene expression, translation, cell morphogenesis, metabolism and regulation of cell cycle progression. We have seen that Hog1 activation induces a transient arrest of cell cycle which is essential for cell survival to high osmolarity. We first showed that SAPK modulates the G1-S and the G2-M transition by targeting specific cell cycle regulated proteins. While in G1 directly targets the CDK inhibitor, Sic1, and down regulates the G1 cyclin expression, delaying S phase entry; in G2, Hog1 pho-

phorilates Hsl1, leading to accumulation of Swe1 preventing the transition into Mitosis. Moreover, we found later that when cells are challenged in S phase, Hog1 activation slows down the replication process presumably to allow the proper transcription of the osmoresponsive gens. However, it still remains unclear the Hog1 mechanism to delay S phase.

P06-145
Silencing of β-catenin expression and its role in human breast cancers

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Breast cancer is the leading cause of cancer death in women worldwide. The regulation of gene expression by steroid hormones plays an important role in the normal development and function in the pathogenesis of endocrine-related breast cancer. In hormonal carcinomas positive correlation between differentiation of the tumor and decline in E-cadherin expression was postulated. It was recently suggested that nuclear receptors (NR) can interact with Akt/cadherin/catenin system. Since breast cancer strongly depend on Akt/NR regulation we attempted to examine the effect of Akt and/or β-catenin inhibitors (type IV, IX and X) as well as silencing of β-catenin (siRNA) on the expression of GSK3β, β-catenin, cyclinD1 and cadherins in established cell lines (MCF7, T47D) with different expression of nuclear receptors and in breast tissue. The high expression of β-catenin and overexpression of MMPs was significantly associated with progression of carcinogenesis in breast tissue. Treatment of the cells with testosterone led to the increased of cell proliferation, migration and the expression of phosphorylated GSK-3P form, resulting in deactivation of the GSK-3b enzyme. Simultaneously translocation of β-catenin into the nucleus was observed which led to increased expression of c-myc, cyclin D1, NR and MMPs in androgen dependent cancer cell line (T47D). Silencing of β-catenin or Akt reduced both ER and AR expression in T47D cell line and ER expression in MCF-7 cells and decreased up to 85% the proliferation of cells while promoting apoptosis. The obtained results indicate that the inhibition of breast cancer cell growth is mediated by the β-catenin protein level what seems to play crucial role in progression of human breast cancer.

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P06-146
β2-chimaerin subcellular localization modulates Rac-mediated responses

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Chimaerins are a family of GTPase-activating proteins that inactivate the Rac GTPase in a diacylglycerol (DAG) dependent manner. This family is composed of four members, α1-and α2-chimaerin that originate from the *CHN1* gene and β1- and β2-chimaerin which are products of the *CHN2* gene. All chimaerin isoforms have a C1 domain, highly homologous to that of PKC isozymes and a catalytic GAP domain. α2- and β2-chimaerins have also an N-terminal SH2 domain, most likely involved in heteromolecular interactions. According to the established model for chimaerin activation, it has been shown that EGF redistributes β2-chimaerin to promote its association with Rac at the plasma membrane. In addition, β2-chimaerin shows a perinuclear and Golgi localization in response to PMA treatment or after stimulation with EGF. These data suggest that the localization of chimaerins in different subcellular compartments may serve as a mechanism that regulates Rac responses in localized regions of the cell. To study the functional effect of the different cellular localizations of chimaerins we have analyzed the activity of β2-chimaerin that we have artificially targeted to cell membrane, Golgi and endoplasmic reticulum. Our preliminary results indicate that β2-chimaerin localized at the plasma membrane participate in the control of actin cytoskeleton and alters cell adherence. This effect is specific of β2-chimaerin localized at the lipid raft, while targeting β2-chimaerin to the disordered membrane does not impact on actin cytoskeleton organization. These preliminary data suggest that chimaerins subcellular localization determines the biological outcome of Rac site-specific signals.

P06-147
The non canonical NOTCH ligand DLK1 modulates macrophage activation

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DLK1 is a member of the EGF-like family of proteins, like the NOTCH receptors and their ligands. In the immune system DLK1 and NOTCH have been involved in myeloid and lymphoid differentiation and in the generation and preservation of embryonic hematopoietic stem cells. We have previously shown that macrophages activated with bacterial lipopolysacharide (LPS) display a higher level of expression and activation of NOTCH1 and a more potent inflammatory response, with increased NF-kB activity. We also demonstrated that DLK1 can interact with NOTCH1 *in vitro*, blocking the binding of the receptor to its canonical ligands and, thus, inhibiting NOTCH1 activity. Here we have investigated the implication of DLK1 in macrophage activation by using peritoneal macrophages from *Dlk1−/−* and *Dlk1+/+* mice. In the absence of *Dlk1*, we observed higher levels of expression of IFNβ and other pro-inflammatory cytokines, like TNFα, IL12 and IL23, when macrophages were activated with agonists of TOLL3 and TOLL4 receptors, such as the dsRNA poli I:C or LPS, respectively. Moreover, some key proteins involved in the synthesis and response to INFβ, like IRF7, IRF1 or STAT1, and others responsible for the activation of IL12 and IL23, such as c-Rel, were increased in macrophages from *Dlk1−/−* mice. In accordance to that, *Dlk1−/−* mice were significantly more sensitive to endotoxic shock in studies of susceptibility to LPS *in vivo*. Furthermore, luciferase assays indicated that DLK1 diminished the activation of an INFβ reporter construct induced by NOTCH1. In summary, the absence of DLK1 seems to potentiate the inflammatory response in murine macrophages in accordance with a higher activation of NOTCH1, suggesting that DLK1 could modulate NOTCH1 effect during macrophage activation.

P06-148
Identification and immunolocalization of MT_1 and MT_2 melatotin receptors in different animal species spermatozoa

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Melatonin is known to regulate a wide variety of physiological processes in mammals. Some of these effects are mediated by the interactions of melatonin with the two melatonin G-protein-coupled receptors MT_1 and MT_2. These receptors have been reported in human and hamster sperm, and our research group has demonstrated the presence and distribution of MT_1 and MT_2 receptors in ram spermatozoa, although they had not been found in other domestic animal species spermatozoa. The melatonin effects on reproductive events have been well established. Given that the melatonin role is more relevant in animals with definitive photoperiod, we have investigated the presence of melatonin receptors in sperm cell of different species. Indirect immunofluorescence has been used to locate receptors 1 and 2 on sperm membrane in donkey, stallion, boar, dog and human. MT_1 seems to be associated with the acrosome and neck in donkey and boar, while MT_2 showed an intense staining on the acrosome in all species and at the post-acrosome and neck in some of them. Furthermore, western-blot identification in protein extracts using antibodies against MT_1 receptor showed a 37 kDa band compatible with MT_1 receptor in all species, a 75 band compatible with MT_1 homodimerization in donkey, stallion, boar and human, and a 32 kDa band compatible with MT_1 activation in all species. In the case of MT_2 receptor, a 37–39 kDa band compatible with MT_2 receptor was showed only in donkey and stallion, two species with high seasonality. In conclusion, we have evidenced the presence of MT_1 and MT_2 receptors in different domestic animal species. The results of western-blotting have suggested that MT_2 receptor might be more involved then MT_1 in the melatonin photoperiod control of seasonal reproduction animals.

P06-149
The pentose phosphate pathway operates in ram spermatozoa

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The oxidative stress induced by reactive oxygen species (ROS) is one of the causes of sperm cryopreservation injury. It has been shown that *in vitro* capacitation results in increased ROS levels, which are dependent of the antioxidant defense that can use the NADPH generated by the pentose phosphate pathway (PPP). However, the functioning of the RPP could not be demonstrated in bull and ram spermatozoa, although its involvement in mouse sperm capacitation has already been shown. The aim of this study was to investigate whether the PPP is operative in ram spermatozoa, and its possible role in capacitation. We evidenced the presence of G6PDH and 6PGDH in ram spermatozoa by indirect immunofluorescence using anti-G6PD and anti-PGD as primary antibodies, and Alexa Fluor 488 chicken anti-rabbit as the secondary one. The results obtained showed the presence of G6PDH in the acrosome of all spermatozoa, while the 6PGDH signalling was restricted to a narrow and intense band at the equatorial region. Enzyme activities were determined by spectrophotometry (340 nm) in ram sperm lysates obtained by nitrogen cavitation (1000 PSI, 15 min equilibration at 4°C) followed by centrifugation (16 800 ***g***, 5 min) and recovery of the supernatant. The activity of both G6PDH and 6PGDH increased from pH 6 and reached the maximum value at pH 8.5. We also determined the Km values of both enzymes for their substrates, and confirmed the presence of these enzymes by western-blotting. Additional assays to evaluate possible changes in the activity of these enzymes during *in vitro* capacitation are currently in progress.

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P06-150
Importance of tissue transglutaminase on the activation of secretory phospholipase A2 in the oxidized LDL-treated vascular smooth muscle cells

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Tissue transglutaminase (TG2) is a Ca^{2+} activated, sulfhydryl-rich protein which crosslinks proteins via ε-(γ glutamyl) lysine bridges and acts as a matrix stabilizer by catalyzing inter- and intra-molecular crosslinks between the extracellular matrix (ECM) proteins. Moreover, TG2 serves as a cell adhesion protein and regulates the cell adhesion mechanism via binding to Syndecan-4 (SDC-4) heparan sulfate proteoglycans. TG2 is found to be highly deposited in fibro-lipid plaques and takes part in the stabilization of the atheromatous plaque. Secretory Phospholipase A_2 ($sPLA_2$) is an upstream regulator of inflammatory responses and modifies the LDL (Low density protein) resulting in the retention and oxidation of LDL which is one of the hallmark of atherosclerosis. Evidences show that TG2 is abundantly expressed by vascular smooth muscle cells (VSMCs) and its activity was found to increase concomitantly with $sPLA_2$ in the atherosclerosis lesions. Given that $sPLA_2$ comprises a binding affinity to heparan sulfate chains (HSCs) on the cell surface and ECM, we hypothesized that $sPLA_2$ by binding to HSCs of Syndecan-4 can be activated by TG2 on the cell surface. The aim of the study is to investigate the role of TG2 as an activator of $sPLA_2$ in the oxidized LDL (ox-LDL) induced VSMCs and the role of SDC-4 in this process. Our results showed that TG2 expression and deposition to ECM was increased in the presence of 50, 100, 150, 200 μg/ml of mmox-LDL, which was found to be in parallel with an increase in the $sPLA_2$ activity. Treatment of 200 μg/ml mmox-LDL induced HAVSMCs with TG2 inhibitor resulted in a 50% decrease in $sPLA_2$ activity suggesting that $sPLA_2$ activation was dependent on transamidation activity of TG2. On the other hand silencing of SDC-4 did not lead to any change in $sPLA_2$ activity in mmox-LDL induced HAVSMCs. Taken together, our data suggests that treatment of HAVSMCs with the mmox-LDL not only leads to an increase in TG2 expression and deposition to ECM but also induce the activation of $sPLA_2$. Besides that, the presence of SDC-4 on the cell surface does not have an effect on the $sPLA_2$ induction.

P06-151
Role of STAT3 in regulation of melanoma cell invasiveness via SERPINA3

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Constitutive activation of Stat3 and Stat5 proteins, is detected in many human tumour cells and cells transformed by oncoproteins that activate tyrosine kinase signalling pathways. Aberrant Stat3 promotes uncontrolled growth and survival through dysregulation of gene expression, including *cyclin D1*, *c-Myc, Bcl-xL, Mcl-1* and *survivin*, thereby contributing to oncogenesis, however mechanisms that associate STAT3 in cancer invasion are poorly understood. The aim of this study was to identify the genes targeted by inhibition of STAT3 expression in melanoma WM239 and T1 cells and to study their roles in tumorigenesis. STAT3 silencing was achieved by siRNA transfection and was followed by global gene expression profiling. Microarray analysis indicated 20 genes up or down-regulated following STAT3 silencing. Functional analysis of these differentially regulated genes revealed that STAT3 silencing significantly affected the expression of a small but interesting subset of genes involved in invasion (*SERPINA3, TSPAN10*), antigen processing and presentation (*CD74*) and chromatin remodelling (*SMARCA2*). QPCR results confirmed the expression level of *SERPINA3, CD74* and *SMARCA2*, with only a slight increase in the level of *TSPAN10*. *SERPINA3* gene encoding a serine protease inhibitor (plasminogen activator inhibitor-*3*) is known to play a part in invasion, but its exact functional role still needs to be explained. Matrigel invasion assay coupled with Laser Scanning Cytometry revealed a reduction in the number of cells migrating through the matrigel after silencing STAT3. The presented results show that STAT3 may play a role in melanoma cell invasion via the regulation of *SERPINA3*.

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P06-152
Tks4 scaffold protein regulates EGF-dependent cell migration

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Tks4 and Tks5 belong to a family of scaffolding proteins recently shown to be involved in podosome formation and cell invasion. In this study, we show that Tks4 is also involved in EGF signaling. It has been found that upon EGF treatment Tks4 translocates to the cell membrane where it associates with the activated EGF receptor and becomes tyrosine phosphorylated. We identified members of the Src family to be the most likely kinases responsible for this phosphorylation. The association between the EGFR and Tks4 is not direct and requires the presence of Src. Since we found prominent Tks4 redistribution to lamellipodia after EGF stimulation we also investigated if Tks4 may play a role in cell migration. Silencing of Tks4 was shown to markedly inhibit HeLa cell migration in a Boyden chamber assay in response to EGF or serum. Our results therefore reveal a new function for Tks4 in the regulation of growth factor-dependent cell migration.

P06-153
Paradoxical activation of the MEK/ERK pathway promotes melanoma invasion and metastasis

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Melanoma is the most deadly form of skin cancer. It accounts for only 10% of cases, but 80% of skin cancer deaths. An important feature of melanoma is its ability to metastasize and this contributes to its poor prognosis. The small G-protein RAS is mutated in ~20% of melanomas and its downstream kinase BRAF is mutated in a further 50% of cases. These oncogenes regulate proliferation and survival through the MEK–ERK cascade, and over the past decade this critical pathway has emerged as an important player in melanoma biology and as a therapeutic target. Selective inhibitors of BRAF such as PLX4032 (Vemurafenib) have a remarkable clinical activity in patients with BRAF mutant melanomas with response rates of approximately 60–80%. Vemurafenib is a BRAF inhibitor and in cells expressing activating BRAF mutations, it blocks activation of the MAPK pathway. However, in cells expressing wild type BRAF where the upstream pathway is constitutively active by mutations in proteins such as RAS, BRAF inhibitors drive the paradoxical activation of the MAPK pathway. Here we investigate the relevance of BRAF inhibition in the context of oncogenic RAS mutations. We show that genetic inhibition of BRAF combined with oncogenic KRAS leads to increased metastasis in a mouse model of melanoma and that BRAF inhibitors increase the invasion abilities of oncogenic RAS mutant cells in a protease-dependent manner through IL8 secretion. We also show that BRAF inhibitors promote melanoma metastasis in an allograft model. We conclude that BRAF inhibitors drive metastasis through paradoxical pathway activation.

P06-154
New Oncogenic drivers in hepatocellular carcinoma development: neddylation and its inhibition as new therapeutical approach

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Hepatocellular carcinoma (HCC) and colon cancer are the fifth and third most common cancers, and the third and second cause of death from malignancy respectively and no effective treatment has been yet identified. HuR is RNA-binding protein frequently

overexpressed in tumors, and its expression in human HCC and colon cancer metastases correlates with the abundance of Mdm2 (murine double minute 2). HuR is stabilized by Mdm2-mediated NEDDylation, ensuring its abundance and nuclear localization. This novel Mdm2/NEDD8/HuR framework appears as pharmacologically amenable for cancer therapy. MLN4924 (Millennium Pharmaceuticals, Inc.) is an investigational drug that inhibits NEDD8 activating enzyme (NAE) blocking Cullin-RING E3 ubiquitin ligases (CRL) and stabilizing cullin substrates. Here we have explored the antitumoral activity of MLN4924 in HCC and colon cancer. We treated hepatoma and colon cancer cells with MLN4924 and we have analyzed its effect in proliferation and apoptosis. MLN4924 induces apoptosis in proliferative cancer cells (increasing Caspase-3 activity and PARP cleavage) and decreasing also the pro-survival Akt signalling pathway and Mdm2 levels. CRL substrates (p21, p27, Cyclin D1 and β–catenin) were accumulated upon MLN4924 treatment. MLN4924 treatment activates JNK signalling (increased levels of phospho-c-jun), and decreases the expression of the tumour suppressor gene PTEN and of the NF-κB targets (Nrf2, p53, Bax and IKKs). Interestingly, the levels of the Neddylated protein HuR were deeply decreased after MLN4924 treatment. More importantly, the overexpression of HuR in hepatoma and colon cancer with a specific HuR-Adenovirus (Xiao et al., 2007), reduced the apoptotic response to MLN4924 making cells more resistant to the NAE inhibitor, and HuR silencing using specific HuR lentivirus (Calaluce et al., 2010) rendered cells more sensitive to the apoptotic response induced by the drug. Summing up, these results highlight the pivotal role of HuR and Neddylation in HCC and Colon Cancer development, which in turn unveils MLN4924 as a potential therapeutic agent for cancer therapy.

P06-155
The HIF oxygen sensing pathways in physiopathology

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Oxygen is an essential element in whole body metabolism of aerobic organisms and accordingly, cell oxidative metabolism needs to be reprogrammed when the availability of oxygen becomes limited. This metabolic adaptation to hypoxia involves a switch from aerobic to anaerobic metabolism that is driven by the hypoxia-inducible factors (HIFs), which are in turn regulated by the prolyl hydroxylase domain proteins (PHD-1, 2 and 3) oxygen sensors. This metabolic reprogramming that attenuates mitochondrial activity acts as a cell autonomous survival mechanism that confers protection to cells against ischemic challenges. Indeed, HIF-dependent repression of mitochondrial function appears to reduce the overproduction of toxic mitochondrial reactive oxygen species and the subsequent oxidative damage typically associated with low flow ischemia or ischemia-reperfusion episodes. Although the impact of HIF-dependent metabolic reprogramming on cell survival following an ischemic challenge has been studied, the role of HIFs in adult whole body metabolism remains poorly understood. Global *Hif1α* gene inactivation in adult mice reduces age-dependent and high fat diet associated body weight and white adipose tissue (WAT) enlargement. Previous studies have shown that WAT suffers regional hypoxia and indeed, WAT becomes progressively hypoxic – and activates HIF1α – as a consequence of its enlargement during body weight gain. Further tissue specific analyses suggest the involvement of a WAT metabolic reprogramming in body weight control upon *Hif1α* gene inactivation. This study establishes an essential role of the oxygen-sensing pathway in body weight control possibly involving a WAT metabolic rewiring.

P06-156
Role for tissue transglutaminase (TG2) in renal carcinoma cell adhesion and migration

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β1 integrins and syndecan-4 (SDC4) heparan sulfate proteoglycans (HSPG) collaborate on activation of focal adhesion formation and turn-over. Cooperatively operating with integrins and SDC4 in the organization of cell adhesion is TG2 as it binds to the HS chains of SDC4. TG2 in association with ITGB1 has been previously shown as a facilitator of drug resistance and metastatic potential in various cancers of the epithelial origin. Here we investigate the role of TG2 in adhesion and migration of primary site (A-498, Caki-2) and metastatic site (ACHN, Caki-1) renal cell carcinoma (RCC) cell lines. Analysis of TG2 transamidating activity showed that cells isolated from the primary site possessed a higher enzyme activity in comparison to the other cell lines, with A-498 demonstrating the highest activity. While Caki-1 cells have shown the highest adhesion and migration potential on fibronectin (FN) and Collagen Type-I (Col1), ACHN cell line had highest cell-adhesion and migration activity on laminin among the four cell lines. Silencing of TG2 resulted in up to a 40% decrease in adhesion and migration of Caki-1 on FN and Col1 as shown by adhesion and transwell migration assays. Similarly, reduction in TG2 expression resulted in a decrease in the adhesion and migration of Caki-2 both on FN and Col1. On the other hand, decrease in TG2 expression did not lead to any significant change in the adhesion of ACHN and A-498 cells on FN and Col1. ACHN demonstrated a greater adhesion and migration potential on laminin (LM) compared to the other cell lines. Adhesion and migration on LM was strongly impaired upon silencing of TG2 in ACHN and Caki-1 cells, while that of Caki-2 and A-498 was found not to be as much. Measurement of collagen biosynthesis showed that A-498 gradually increased collagen synthesis for 48 hours while ACHN and Caki-2 demonstrated turnover after the first 24 hours. Synthetic rate of collagen was gradually decreased at 24 and 48 hours in Caki-1 which may be an indicative of high metastatic phenotype. Treatment of RCC cell lines with TG2 inhibitor suggested that the biosynthesis of collagen was affected by TG2 activity possible through activatory effect of the enzyme on TGFβ.

P06-157
The effect of PKCeta expression in ER stress-induced autophagy is not mediated by JNK pathway

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The endoplasmic reticulum (ER) of eukaryotic cells is the site of synthesis, folding and posttranslational modifications of membrane and secretory proteins. Several environmental, genetic and metabolic conditions may alter protein-folding reactions in the ER, resulting in ER stress which induces the activation the unfolded protein response (UPR). Autophagy, as a cytoprotective response to stress stimuli, can help cells to cope with ER stress through the elimination of unfolded proteins and inhibition of ER stress-induced cell death. The novel protein kinase C (PKC) eta isoform is a serine/threonine kinase involved in a variety of

cellular responses. Since PKCeta is localized in the ER and the Golgi apparatus, we aimed to investigate its effect on cell responses to ER stress. The role of PKCeta in autophagy induced by the ER stressor, thapsigargin (TG), was examined in MCF-7 breast cancer cells inducibly expressing PKCeta under the control of a tetracycline responsive promoter. The level of the autophagic marker LC3-II was reduced by the inducible expression of PKCeta in compare to control cells in response to TG treatment. Additionally, an increased level of LC3-II was also demonstrated in response to TG in shRNA PKCeta knocked-down cells compared to control cells, implying that PKCeta expression affects the autophagic pathway. JNK pathway is one of the UPR pathways activated in response to ER stress. In response to TG, PKCeta expression reduced JNK activity as the level of phosphorylated c-Jun was elevated in control cells compared to PKCeta-expressing cells. However, JNK pathway doesn't affect autophagy and apoptosis, since inhibition by JNK specific inhibitor (SP600125) in TG-treated cells has no effect on LC3-II autophagic marker and PARP-1 apoptotic marker, respectively. Taken together, the effect of PKCeta expression on autophagy is not mediated via JNK signaling pathway, probably involved different signaling pathways. As ER stress and autophagy are implicated in several diseases, the role of PKCeta expression in these processes could be of therapeutic value.

P06r-158
A novel motif mediates binding of dual-specificity phosphatases to the yeast cell wall integrity MAPK

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Mitogen-activated protein kinase (MAPK) pathways, widely conserved among eukaryotes, constitute very tightly regulated signaling modules that transduce extracellular stimuli into appropriate cellular responses. MAPKs are activated through phosphorylation in specific threonine and tyrosine residues. Therefore, serine-threonine, tyrosine and dual specificity phosphatases (DSPs), which dephosphorylate both type of residues, play an essential role in the negative regulation of these pathways. We are particularty focused on the regulation of the Cell Wall Integrity Pathway (CWI) in *Saccharomyces cerevisiae* by DSPs. Among them, Msg5 and Sdp1 have already been described as negative regulators of the CWI MAPK Slt2. Here we report the identification of a novel motif (IYT) located in the N-terminal non-catalytic regulatory domain of Msg5 and Sdp1 that mediates its binding to Slt2 as well as to the pseudokinase Mlp1. This interaction is independent of the MAPK common docking (CD) domain in contrast to the D domain-dependent mechanisms that participate in the binding of Msg5 to mating MAPKs (Fus3 and Kss1). Since this motif IYT is conserved in other orthologs from yeast species such as the DUSP Pmp1 in *S. pombe*, we suggest that it might participate in a novel conserved binding mechanism.

P06-159
An inactive receptor for epidermal growth factor (EGFR) interacts with HER-2 to induce proliferation in cervical cancer cell lines

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The HER family receptors have an important role controlling cell growth and differentiation. The family consists of 4 receptors: ErbB1, ErbB2, ErbB3 and ErbB4, which establishes an extensive signaling network that controls diverse biological processes such as embryogenesis and oncogenesis. Members of the family have been implicated in malignant transformation and are over expressed in a variety of human tumors. We have found that CALO and INBL cell lines, derived from Mexican patients diagnosed with epidermoid cervical carcinoma composed of non keratinized large cells from metastatic and non metastatic tumors, express an unphosphorylated form of the EGF receptor and a constitutively active HER-2 receptor. Therefore, the aim of this study was to address receptor dimerization in order to induce proliferation signals in cervical cancer cells. We demonstrate that EGFR and HER-2 are forming heterodimers in CALO and INBL cells. By inhibiting HER-2 phosphorylation by specific tyrosine kinase inhibitors, tyrphostins, the proliferation of both cell lines is inhibited. It is very likely that the EGFR is an inactive tyrosine kinase and that is interacting with HER-2 to potentiate proliferation signals through a signaling pathway different from the canonical EGF pathway. The unraveling of the mechanisms of EGFR and HER-2 involvement in cervical cancer may lead to more effective drugs and therapeutic strategies.

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P06-160
Conserved regulatory mechanisms of dopaminergic terminal differentiation

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Dopamine (DA) is one of the main neurotransmitters in the brain. Dopaminergic neurons are very well conserved in evolution, all animals express the same battery of enzymes and transporters to synthesize and release DA. We call this set of genes the DA pathway genes. The identification of the regulatory logic that controls the expression of the DA pathway genes will give us the clues to understand the dopaminergic terminal differentiation. Taking advantage of the DA phylogenetic conservation, we have used the model organism *C. elegans* to study the regulation of the DA pathway genes. Our previous work showed that AST-1, a transcription factor from the ETS family, directly activates the expression of all DA pathway genes in all dopaminergic neurons in the worm. This regulation is also conserved in mammals: ER81, the mammalian homolog of AST-1, also plays a role in the differentiation of the mouse olfactory bulb dopaminergic neurons (Flames N., 2009). However, neither AST-1, nor its corresponding binding sites, are sufficient to activate the DA pathway genes suggesting that other factors are needed for the terminal differentiation of dopaminergic neurons. Using a combination of forward genetics and promoter analysis, we have now identified two other types of transcription factors that act together with AST-1 in the coordinated activation of the DA pathway genes. Our promoter analysis reveals the presence of functional homeodomain and PBX binding sites in all DA pathway genes. Moreover, we have isolated several mutant alleles of the Distal-less homeodomain transcription factor ceh-43 that show defects in DA neuron terminal differentiation. Analysis of animals lacking the PBX factor ceh-20 also shows defects in the differentiation of the dopaminergic neurons. Interestingly, Dlx2, the mammalian homolog of ceh-43 has already been shown to play a role in dopaminergic specification of the mouse olfactory bulb. This finding, together with our previous work on the mouse ETS factor Er81 suggest that the regulatory logic of dopaminergic terminal differentiation is evolutionary conserved.

P07 – Developmental Biology

P07-1
Effects of *Proteus vulgaris* OX19 on the spleen cells of rabbit

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The effects of *Proteus vulgaris* OX19 on spleen cells of rabbits were investigated. Control group (n = 5) and *Proteus* treated group (n = 5) New Zealand male rabbits were used in this study. Bacteria were injected to the rabbits in five days periods with increasing dosages for one month. Spleen tissues were removed, fixed and embedded in araldite, cut with an ultramicrotome. Thin sections were examined by Transmission Electron Microscope (Jeol 100CXII). Ultrastructural changes were observed in spleen tissue due to the antigenic stimulation of bacteria to the rabbits. Spleen cells of control group were in normal structure. Spleen cells of *Proteus* treated group displayed structural changes as regard to the control group in electron microscopic examinations. Chemotaxis of macrophages and presence of phagocytic vacuoles were observed. Fine structure of lymphocytes revealed mitotic activity. In addition, loss of chromatin material, increase in the number of mitochondria and dilatations in perinuclear space were significant. Interaction of lymphocytes and macrophage cells were noteworthy.

Keywords: ultrastructure, *Proteus vulgaris*, spleen, rabbit, transmission electron microscopy

P07-2
Somatic embryogenesis and plant regeneration of *Lilium bosniacum*, an endemic Bosnian species

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The objective of the present study was to determine the optimal concentrations of plant growth regulators in the medium for lily regeneration from leaf and bulb seedling explants of an endemic lily *Lilium bosniacum*, and to relate morphological responses with photosynthetic pigments, protein content and peroxidase activities. For these purposes leaf and bulb explants were cultivated on different concentrations of BA and 2.4-D. After 75 days from culture initiation the morphological and biochemical responses of plantlets were assessed. Morphological parameters of lily plantlets were affected by cultivation conditions, ea. ratio of used growth regulators, as well as by explant type. Growth of bulblets was strongly affected by used treatment, leading to significant increase in bublet number (on treatments 2.0 mg/l 2.4-D + 1.0 mg/l BA for basal leaf explants). An increase in peroxidase activity appeared to be a good indicator for somatic embryogenesis and organ differentiation for *L. bosniacum*, as noticed on some used treatments.

P07-3
Genomic architecture, gene regulation and human diseases

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The generation of distinctive cell types that form different tissues and organs requires precise, temporal and spatial control of gene expression. This depends on specific cis-regulatory elements distributed in the non-coding DNA surrounding their target genes that became active or inactive at particular developmental stages. On the top of this, the 3D structure of the chromatin plays an essential role in facilitating the access of such cis-regulatory elements to particular promoters. In this seminar, I will discuss the importance of the chromatin architecture and the dynamic of cis-regulatory elements during development and its implication in human diseases and genome evolution.

P07r-4
Compared evolution of photoperiodic signaling in green algae and plants

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Photosynthetic organisms are able to integrate external and internal signals to synchronize important biological functions, so that, vital process like flowering, take place in the best environmental conditions. The day-length dependent or photoperiodic pathway, is the most conserved flowering signaling system in spermatophyte plants (1). This way, the regulation of the *CONSTANS* gene (*CO*) is the key to trigger the activation of the florigen *FLOWERING LOCUS T* (*FT*) that induces the flower differentiation (2).

Our research group has showed that the photoperiodic pathway preceded in evolution to vascular plants and can be found in unicellular photosynthetic organism such as chlorophyceae algae (2). Recent works performed in *Chlamydomonas reinhardtii CO* homologous gene (*CrCO*) support this hypothesis (3). At the moment, our research is focused in the discovery of new conserved genes of the photoperiod pathway to know the original molecular mechanism involved in the process. The *CO* transcriptional inhibitors in *Arabidopsis* called *CYCLING DOF FACTORS* (*CDFs*) are a family of 4 DOF type transcription factors, while there is a single copy in *C. reinhardtii* (*CrDOF*). By using classic techniques of mutant generation, phenotypic analysis, biochemistry, transcriptomics and cellular biology of both models organisms, we will show evidences supporting that *CrDOF* could be another conserved gene of this primitive photoperiodic pathway.

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P07-5
C-RAF deficiency causes cochlear abnormalities and profound sensorineural deafness in the mice

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Insulin-like growth factor I (IGF-I) is fundamental for neurogenesis and neuronal differentiation during inner ear development. IGF- I deficiency is associated with deafness in man and mice (1). IGF-I binds to its high affinity receptor and activates downstream signalling as the RAF-MEK-ERK pathway. RAF kinases are essential for cell proliferation, survival and differentiation during development and in the adult tissues homeostasis. RAF proteins have redundant but also specific cellular and tissular functions. In the developing chicken inner ear the activation of C-RAF and B-RAF are critical for otic neurogenesis (2). To further study the role of RAF kinases in the auditory receptor, we have analyzed C-RAF mRNA and protein expression patterns in the mouse inner ear along development. Our results show that C-RAF is differentially expressed and that the protein is active and able to phosphorylate downstream substrates. To explore its functional relevance we have studied the phenotype of the *Raf1*$^{-/-}$ null mouse. *Raf1*$^{-/-}$ mutants present an all-frequency profound sensorineural hearing loss with a mean auditory threshold of 90 dB SPL. The study of the general cochlear cytoarchitecture indicates that the main structures and cell types have been formed, although the expression of proteins essential for hearing is altered. Thus the levels of the Kir4.1 potassium channel in the stria vascularis are reduced in the *Raf1*$^{-/-}$ null when compared to the wild type littermates. In summary, these results show that C-RAF is expressed in the developing cochlea and that its activity is essential for the onset of hearing. Acknowledgements: RdI holds an 'Autónoma University of Madrid' fellowship.

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P07-6
Identification of brain abundant proteins BASP1 and GAP-43 as components of mouse oocytes and preimplantation embryos

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Similarity of calcium-activated signaling in oocytes and axon terminals prompted us to investigate the presence of the presynaptic proteins BASP1 and GAP-43 in oocytes. Using immunocytochemical techniques combined with confocal microscopy, we have revealed that both BASP1 and GAP-43 are present in mouse oocytes, zygotes and preimplantation embryos. As in neurons and other cell types, BASP1 was found to be localized at the plasma membrane and actin cortex of the oocytes and zygotes. GAP-43, which is mainly considered as postmitotic membrane marker of nerve cells, was shown to reside at the meiotic spindle microtubules in the metaphase II (MII) oocytes. At the spindle poles (centrosomes) and microtubule organizing centers in the cytoplasm, GAP-43 is co-localized with γ-tubulin. In oocytes, GAP-43 is subject to phosphorylation by protein kinase C, as shown with antibodies to Ser41-phosphorylated form of the protein. After completion of the meiosis, GAP-43 is present in the midbody and the second polar body. In early embryos until the blastocyst stage, GAP-43 is present in the nuclei and cytoplasm. The expression of BASP1 and GAP-43 in oocytes at the protein level was confirmed by electrophoresis and western blotting. Microinjection of bovine BASP1 (but not GAP-43) in the cytoplasm of MII oocytes induced their exit from MII arrest followed by parthenogenetic embryo development. This suggests participation of BASP1 in oocyte activation, probably through regulation of local concentration of polyphosphoinositides in the plasma membrane. GAP-43 localization at the spindle and centrosomes was recently shown in asymmetrically dividing neuronal progenitors. Therefore we suggest that GAP-43 may be involved in regulation of spindle orientation and oocyte polarity.

P07-7
Role of hypoxia inducible factors in pancreas formation

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The main effectors of the cellular response to hypoxia are the transcription factor hypoxia-inducible factors(HIFs) proteins. Under normal oxygen levels (normoxia), the HIF factors are degraded by the von Hippel-Lindau (VHL) tumor suppressor-containing ubiquitin ligase complex. Under low oxygen(hypoxia) conditionsthe HIF-α subunitsare not degradedjoining the beta subunit of HIF transcriptional complex that then translocate to the nucleus to control the transcription of a number of genes the help the cell to cope with the stress, including genes involved in cellular energy metabolism, glucose metabolism and angiogenesis. Recent studies have uncovered a new role of the hypoxia-HIF pathway in pancreatic beta cell formation and function. Overactivation of HIF pathway through inactivation of the VHL gene results in impaired adult beta cell function. However, it is not completely clear whether these effects are mediated by HIF1-α or HIF2-α.

To gain insight into the differential role of HIF1-α and HIF2-α in islet function, we are using Cre-loxPtechnology to overproduce a modified version of both HIF1-α and HIF2-α. The HIF variants harbor mutations that make them not recognizable by the VHL complex, and therefore are accumulated under normoxia conditions. We have used a pancreas-specific Cre line to determine the effects of HIF1-α and HIF2-α accumulation during embryonic development.

Our results have revealed that HIF accumulation results in impaired pancreas formation, particularly in the exocrine cell lineage. Endocrine cell formation is also affected as islet mass is decreased in HIF-overproducing mice. Furthermore, the remaining islets show hypervascularization and abnormal distribution of alpha and beta cells. These results suggest a possible role of the HIF pathway in embryonic pancreas formation.

P07-8
Molecular mechanism of embryonic stem cell self-renewal regulated by the histone protein H2AX

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Self-renewal is the process through which an embryonic (ES) or adult stem cell generates one or two daughter stem cells with developmental potential similar to the mother cell. Although, several mechanisms that regulate self-renewal have been described, the detailed molecular processes are still not fully understood. Recently, it has been shown that the histone protein H2AX regulates self-renewal by inducing a slow proliferation of ES, as well as, adult stem cells of the sub ventricular zone of the hippocampus. H2AX is involved in mechanisms of DNA damage response, that are mechanisms of DNA repair, in particular, H2AX recruits several proteins and it induces the formation of a big complex having the function to check and repair the DNA damage. Interestingly, it has been shown any DNA damage during the process of ES self-renewal regulated by H2AX.

In order to understand how H2AX regulates ES self-renewal, we have performed a proteomic analysis in which we identified 30 proteins associated with H2AX. *In silico* analyses, using databases of known and predicted protein-protein interactions, show that the identified proteins are present in known chromatin remodeling complexes, such as the SWI/SNF and the FACT complex. Moreover, nine of the identified proteins are known direct interactors of H2AX, confirming the accuracy of the result. In addition, we confirmed the proteomic analysis results by reciprocal immunoprecipitations.

The analyses show for the first time a detailed molecular pathway involved in the regulation of ES cell self-renewal and it describes in depth the new role of H2AX in controlling ES cells self-renewal.

P07-9
Age-related changes in the ultrastructure of the rat ovaries

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The reproductive organs of females exhibit a rate of ageing that is much faster than that of the other body systems with decrease in fecundity. The objective of this study was to determine theage related changes in the ultrastructure of the rat ovaries. Thirty female Wistar-albino rats were used. These rats were divided into five groups according to age: young (22 days), prepubertal (4 weeks), adult (10 weeks), premenopausal (8 months) and old (2 years). Six rats allocated in each age group. The left ovaries were removed, and tissues were extracted and examined using electron microscopy. When the structural changes evaluated, it is observed that the number of annular lamellas and balbiani particles diminished by ageing. During the early stages of development zona pellucida was in a uniform thickness but it appeared to be floppy during the menopause.

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P07-10
Role of glial cell line-derived neurotrophic factor in embryonic pancreas colonization by neural precursors

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Recent studies have suggested that endocrine pancreas formation is regulated by signals from the neural crest cells that migrate and populate the gut with neural and glíal cells during embryonic development. Glial Cell Line-Derived Neurotrophic factor (GDNF) plays a critical role in the development and survival of the enteric nervous system and both, *in vivo* and *in vitro* experiment, have shown that GDNF overproduction promotes beta cell survival and proliferation. We have confirmed GDNF expression soon after the páncreas evaginates from the foregut. This expression persist during páncreas embryonic development in a dynamic fashion, correlating with endocrine progenitor domains in the pancreatic epithelium, and switches off after birth. GNDF was inactivated in developing pancreatic epithelium using the Pdx-1-Cre transgenic mouse line to test the role of endogenous expression on páncreas development. No changes in islet architecture and mass were observed, demostrating that GDNF is dispensable for islet formation. Hovewer, a profound loss of neural cells was found in mutants mice as determined by inmunohistochemistry staining for neural and glial markers. Our análisis of embryonic pancreas and *ex vivo* experiments demostrates that GDNF is acting as a neurotrophic factor for neural crest derived cells and indicates that migration of neural progenitor cells is compromised in mutant pancreas. These data indicates that the GDNF expression during pancreas embryonic development is essential for neural colonization but dispensable for páncreas formation. So, we believe the páncreas especific GDNF mutant mice might be a useful tool to analyse neural-islet interactions in adulthood.

P07r-11
Role of the posttranslational regulation of CONSTANS protein in photoperiodic flowering in *Arabidopsis thaliana*

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The photoperiodic pathway regulates most of the flowering transitions in plants. In Arabidopsis, the fine regulation of the transcription factor CONSTANS determines its flowering time activating the production of the florigen FT in the vascular bundles (1). CO is controlled at several levels: from the circadian regulation of his transcript to the modification of its stability dependent on photoreceptors and the proteasome (2).

The protein CO contains three characteristic domains with particular functions: interaction with other proteins, import to the nucleus and the activation of transcription. That maintains the protein CO stable and active at particular moments (3,4).

Arabidopsis has established a complex mechanism to control the presence of the transcripts and the stability of the protein thanks to protein complexes regulated by hormones and photoperiod (5).

In this work we have identified components of the molecular complexes by diverse experiments to study other CO interactors.

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P07-12
The transcription factor Mef2A (SrfC) regulates cell differentiation in *D. discoideum*

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The amoeba *D. discoideum* has been used as model system in many developmental studies because of its capacity to form multicellular structures, called fruiting bodies, formed by a cellular stalk and, on top of it, a sorus where most of the cells differentiate into spores. Transcription factors of MADS-box family play important roles in cell differentiation and development of diverse organisms, including animals and plants. *D. discoideum* encodes four transcription factors of the MADS-box family, two of them are more similar to animal SRF (Serum Response Factor) factors, and the other two more similar to animal Mef2 (myocyte enhancer factor 2) factors. The structure and function of the gene coding for one of the two Mef2 homologous factors, Mef2A, is presented in this communication. Mef2A is expressed in vegetative cells and in the prespore region of developing structures from two alternative promoters. Developmental expression is markedly induced from 4 hours of development. The biological function of the gene has been studied through the generation of mutant strains where the gene has been partially deleted. Mutant strains showed reduced growth feeding on bacteria, but not in axenic media. These strains also showed impaired formation of slug structures and produced a reduced number of spores. Development of the mutant strains was further studied through analysis of the expression of cell-type-specific genes, the generation of chimeric structures between wild-type and *mef2A* mutant cells, '*in vitro*' differentiation assays and mRNA-sequencing experiments. In summary, the results obtained indicate that Mef2A participates in the determination or differentiation of presopore cells, and a population of prestalk cells, although it is not absolutely required for these processes.

P07r-13
PTEN deficiency enhances estradiol proliferation on endometrial epithelial glands

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Loss of tumour suppressor PTEN is the most common molecular alteration found in endometroid endometrial cancer. However, the complex relation between loss of PTEN and growth factor such as IGF of EGF and hyperstrogensim in the development of endometrial cancer is still controversial. Here, using a 3D glandular cultures of PTEN+/+ and PTEN+/− endometrial epithelial cells, we have investigated the effects of EGF, Insulin/IGF and estradiol in the development of endometrial carcinoma. We have previously demonstrated that 3D cultures of endometrial epithelial cells require EGF and Insulin/IGF to display proliferative response. Here, we demonstrate that chronic exposure to estradiol induces proliferation of 3D glandular cultures. Moreover, we show that proliferative effects of estradiol are require the presence of Insulin/IGF and EGF. Withdrawal of either EGF or Insln/IGF completely abolishes estradiol-induced poriferation. PTEN+/− exhibited only a slightly increase in proliferation over PTEN+/− cultures in presence of EGF and Insulin/IGF. However, further addition of estradiol caused a exaggerated proliferation of PTEN+/− cultures, leading to formation of complex structures that those observed in endometrial hyperplasia or carcinoma. Noteworthy, induction of proliferation by estradiol also requires the presence of Insulin/IGF and EGF; otherwise proliferative effects of estradiol are completely abolished. In summary, we demonstrate that EGF and Insulin/IGF prime endometrial epithelial cells to proliferative effects of estradiol, and PTEN loss results in enhanced responsiveness to these combination resulting to the development hyperplasia o endometrial cells in culture.

P07-14
A PHABULOSA/cytokinin loop controls root growth in *Arabidopsis*

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An extraordinary characteristic of plants is the ability to modulate their growth in response to environmental conditions. In this context, phytohormones act as key factors quickly modulating plant growth. Cytokinin (CK) is a phytohormone implicated in different developmental pathways including cell division in the shoot and cell differentiation in the root (1). Despite the key role that CK has during plant development, little is known about how CK biosynthesis is regulated and maintained. HD-ZIPIII transcription factors are essential for the development of both shoot and root (2, 3). Here we show that gain- and loss-of-function mutants of *HD-ZIPIII*s display characteristics that resemble plants in which the levels of CK are increased and reduced, respectively. We have obtained evidence indicating that theHD-ZIPIII protein PHABULOSA (PHB) rapidly controls the rate of CK production to control root development.

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P07-15
Sumoylation in the developing neural tube

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Proliferation and differentiation are critical processes during development. In this context, the developing neural tube is a major field of study due to potential application of the obtained results in the treatment of central nervous system lesions and neurodegenerative diseases. The understanding of the involved mechanisms depends on the identification and characterization of the regulatory events and signaling pathways that lead to the correct progression of development. A signaling system that increases its relevance, specially concerning transcriptional regulation, is the protein modification by SUMO. SUMO (Small Ubiquitin-like MOdifier) is a small polypeptide, similar to ubiquitin, attaching covalently to other proteins as a post-translational modifier. The modification process shares several similarities with ubiquitination, as the presence of an activating E1 enzyme and a

conjugating E2 enzyme, which transfers SUMO to the target protein. In most of the cases, this process is facilitated by an E3 ligase. To this point, the family of proteins PIAS has been widely studied. Sumoylation has been related to transcriptional regulation, nucleus-cytoplasm transport, genomic and protein stability and enzymatic modulation. In the neural landscape, modification by SUMO has been studied in synapses and neurodegenerative diseases, but a role in neural development is still unclear. Taking into consideration that SUMO modification is essential in eukaryotes and relevant for transcriptional regulation and many other functions, its implication in neural development is needed. In this work, we have investigated the role of sumoylation during the development of the vertebrate central nervous system by electroporation of SUMO molecules and ligases of the PIAS family.

P07-16
The histone variant macroH2A regulates the commitment of embryonic and adult stem cells

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The most striking epigenetic alteration occuring on the level of the nucleosome is the complete exchange of a canonical histone H2A for a macroH2A variant. Here, we provide first insight in the function of this unique histone variant in embryonic and adult stem cells. In mouse embryonic stem cells macroH2A1 and particularly its 1.2 splice variant is the predominantly expressed form of macroH2A. During differentiation levels of macroH2A1 but also of macroH2A2 increase. Knockdown of macroH2A1 does not affect self-renewal of embryonic stem cells but limits their capacity to differentiate. Loss of macroH2A1 interferes with proper inactivation of pluripotency genes and results in a reduction and/or delay in the activation of differentiation genes most of which are direct target genes of macroH2A. In addition MacroH2A1-deficient mES cells form defective embryoid bodies that are reduced in size and lack stage-specific cavitation. The requirement of macroH2A1 for proper differentiation is further illustrated *in vivo* by the massive expansion of immature tissue in macroH2A1-deficient teratomas. Furthermore, in the heterogeneous culture of primary human keratinocytes macroH2A1 levels negatively affect the self-renewal capacity of the pluripotent compartment. Taken together these results identify macroH2A1 as a critical chromatin component regulating the delicate balance between self-renewal and differentiation of embryonic and adult stem cells.

P07-17
Spindle assembly checkpoint-related meiotic defect in oocytes from LT/Sv mice is cytoplasmic and diminishes in older females: a possible link to maternal-age-related aneuploidy

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Spindle Assembly Checkpoint (SAC) ensures proper segregation of chromosomes by blocking the cell at metaphase and thus delaying anaphase onset until all kinetochores are properly attached to spindle microtubules. The high frequency of errors in mammalian female meiosis, especially in older individuals, may suggest that SAC efficiency in oocytes is impaired. Oocytes from mouse strain LT/Sv arrest at first meiotic metaphase (MI) due, as recently reported, to enormously prolonged activity of SAC. We studied the dynamics of cyclin B1-GFPdegradation, the process which is a measure of SAC activity, in chromosomal and achromosomal halves of LT/Sv oocytes and confirmed that MI arrest in LT/Sv oocytes is SAC-dependent. Transferring the oocyte nucleus from LT/Sv oocytes into the enucleated oocytes from wild type mice resulted in completion of meiosis one, whereas the introduction of the wild type oocyte nucleus into LT/Sv cytoplasm resulted in MI arrest. Thus, SAC-activating defect in LT/Sv oocytes is cytoplasmic, yet can be rescued by foreign cytoplasm. Our results may help to define the etiology of human infertility related to oocyte metaphase I arrest indicating the involvement of SAC as likely candidate, and point to nuclear transfer as the possible therapy. Finally, we found that most oocytes isolated from old LT/Sv females complete the first meiosis. Such, age-dependent disappearance of LT/Sv phenotype raises the possibility that SAC in mammalian oocytes may weaken as female ages in this way contributing to the age-related aneuploidy.

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P07-18
Human synovial cells as a source of mesenchymal stem cells and pluripotent stem cells

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Induced pluripotent stem cells (iPSCs) generated from patients provide invaluable resources for regenerative medicine. In this study, we cultured synovial cells isolated from two 71-year-old females with advanced osteoarthritis and determined their mesenchymal stem cell (MSC)-like characteristics by observing the expression of specific markers including CD14(−), CD19(−), CD34(−), CD45(−), CD44(+), CD51(+), CD90(+), CD105(+), and CD147(+) as well as their multilineage differentiation potential. Microarray analysis of human MSCs (hMSCs) and human synovial cells (hSCs) determined their unique and overlapping expression patterns. We show that expanded hSCs can be reprogrammed into induced pluripotent stem cells (iPSCs) by ectopic expression of four transcription factors (Oct4, Sox2, Klf4, and c-Myc). STR genotype analysis confirmed that hSC-derived iPSC lines were derived from the donor cells. The pluripotency of hSC-derived iPSCs resembling human embryonic stem cells (hESCs) was confirmed by the hESC-like morphology, expression of pluripotency markers, global gene expression profiles, epigenetic status, normal karyotype, and *in vitro* and *in vivo* differentiation potential into the three germ layers. Undifferentiated hSC-derived iPSCs can be maintained under human feeder culture conditions for extended period of time. Our work demonstrates that patient-derived synovial cells are an attractive source of MSCs as well as iPSCs and will advance cartilage tissue engineering and cell-based model systems of cartilage defects.

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P07-19
Base excision DNA repair in the embryonic development of sea urchin, *Strongylocentrotus intermedius*

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DNA repair is a system of several enzymatic pathways that reverse DNA damage occurring in living cells through the action of numerous endogenous and environmental factors, including ultraviolet light and ionizing radiation. Base excision DNA repair is the pathway that cleanses DNA of oxidized, alkylated and deaminated nucleobases and apurinic/apyrimidinic (AP) sites. DNA repair should be most important for actively proliferating cells; therefore, studies of this process in the embryonic development of animals and plants are of great interest. Sea urchins belonging to the *Strongylocentrotus* genus are convenient objects for such studies, since their development is known to a great detail, and the genome of one species, *S. purpuratus*, is sequenced. We have used oligodeoxyribonucleotide substrates containing DNA lesions in a defined position to study the activity of several base excision repair enzymes at different stages of embryonic development of the sea urchin, *S. intermedius*. All developmental stages contained uracil-DNA glycosylase and A-Pendonuclease activities; these two enzymes represent two initial stages of base excision repair of uracil, the major deaminated nucleobase in DNA. The relative uracil-DNA glycosylase activity increased with the advancing development, especially after blastula hatching and pluteus formation. The same was true for APendonuclease activity; however, the specific APendonuclease activity was overwhelmed by non-specific nucleases at the last stages of development. These increases are likely due to the increase in the number of proliferating nuclei and increased exposure of the embryo to aggressive environmental factors after hatching. The activity of 8-oxoguanine-DNA glycosylase, the enzyme that initiates repair of oxidized purine nucleobases, remained low at all stages of development, possibly reflecting the need for stimulation by other base excision repair factors or low oxidative stress in the embryo. The activity of DNA polymerases at the same developmental stages was studied. Although the relative DNA-polymerase activity decreased during the development, there was a great rising at the stage of blastula hatching. Overall, our results suggest that quick repair of damaged bases is important for actively proliferating cells during embryonic development.

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P07-20
Epigenetic modifications during mouse oogenesis: immunofluorescence analysis of HIRA, histone H3.3, as well as histone H3K36 and H3R17 methylation in prophase mouse oocytes

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During the growth phase of prophase oocytes a set of epigenetic modification of the chromatin occurs, called reprogramming, ensuring that genome of the maternal gametes acquires the ability to support full embryonic development. Chromatin structure and function are controlled by distinct patterns of epigenetic modifications: covalent modifications of histones or by utilization of histone variants. Histone methylation is so far the most complex modification and methylation of the lysine and arginine residues has important consequences for chromatin structure and gene regulation. Tri-methylation of histone H3K36 is connected to the process of active transcriptional elongation and it is reported to be enriched in the 3′ end of active genes. Methylation of histone H3R17 has been linked to transcriptional activation and H3R17me2 is implicated in the regulation of pluripotent genes during the early mouse development. Both these modifications are strong candidates to contribute to reprogramming. Histone H3.3 is a variant of histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Histone H3.3 is deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. HIRA is a histone chaperone that preferentially places the variant histone H3.3 in nucleosomes and is consider to play a role in transcriptional regulation and chromatin and histone metabolism. Through immunofluorescence applying antibodies recognizing histone H3 methylation at K36m3 and R17m2 we have found that both these residues are methylated in the mouse oocytes at the prophase of the first meiotic division. However, a significant decrease of the methylation of both these residues occurs at the end of the growth phase when the oocytes enter the period of transcriptional silencing and coincident with the distinct change of chromatin organisation. By immunofluorescence applying antibodies recognizing histone H3.3 variant we have found that it is present during growth phase and in the fully grown mouse oocytes. At present we also characterize HIRA protein during the phases of oocyte growth by evaluation of its amount and distribution. The results will be presented.

P07-21
Cytokinin action during senescence strongly depends on their concentration and incident light

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The cytokinins are one of the most well-known and best-understood groups of plant hormones. While cytokinins are generally associated with a retardation of senescence ('ageing of plant or plant parts'), there is also some evidence that cytokinins can accelerate senescence. This reversal of cytokinin influence use to be linked to high cytokinin concentrations and their 'toxic' action. Our experiments with detached barley leaves and two cytokinins (*meta*-topolin and benzyladenine) showed that light can also have a profound effect on the influence of cytokinins on the course of senescence. In dark senescing leaves, cytokinins had the expected positive effect (i.e. slowed down decreases in chlorophyll content and F_V/F_M) in all concentrations used. However, the positive effect of the cytokinins decreased with increasing light dose, and at certain point changed to negative ones (i.e. cytokinins accelerated the decreases in both parameters). This trend was more pronounced in case of higher cytokinin concentrations. The acceleration of senescence by cytokinins was further enhanced by exposing the leaves to higher light doses.

On the basis of our observation we propose a model that correlates the effects of increasing cytokinin concentrations with their influence on the onset of senescence and extended this

model to account for the influence of light. We conclude that the effects of light and cytokinins are additive in some respects, but when their combined stimuli exceed a certain threshold, the resulting effect is almost directly opposed to those induced by exposure to their lower levels. This model explains some of the apparently contradictory activities of cytokinins occurring in the literature.

P07-22
Primary high density chondrogenic cultures versus a cell line based chondrogenic model

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Introduction: One of the main murine models to conduct research on cartilage differentiation is the embryonic mesenchymal cell line, C3H10T. Under high density (HD) culturing conditions, with the addition or the overexpression of the growth factor BMP2 chondrogenesis can be induced. Another possible method is establishment of primary HD cultures from chondrogenic cells isolated from limb buds of mouse embryos.
Objectives: Here we attempt comparing the differentiation processes occuring in the two different models including results from non-overexpressing C3H10T cultures as a control for the treated cells.
Methods: We carried out our gene expression studies by the means of RT-PCR reactions, using primers designed on sequences available in the NCBI database. Dimethyl methylene blue, Alizarin Red and Oil Red O stainings were applied to visualize differentiation in various mesenchymal-derived directions (cartilage, bone and adipose tissue, respectively).
Results: In the BMP2-overexpressing model appearance of metachromatic extracellular matrix takes approximately 8 days, while in the case of the primary cultures the same process occurs in a shorter culturing period. We confirmed the differentiation of these cultures in chondrogenic, osteogenic and adipogenic directions.
Conclusions: Overall, the results strongly suggests the primary HD cultures are more focused in the chondrogenic direction, than the HD cultures derived from the cell lines. Metachromatic extracellular matrix is deposited at a faster rate, expression levels of osteogenic and adipogenic markers is depressed compared to the other models involved in the experiments.
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P07-23
SoxD genes and the control of cell proliferation and patterning formation in spinal cord development

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During the development of the nervous system, a vast number of different neurons and glial cells are generated from a small population of self-renewing progenitor cells. At the same time that neural progenitors exit the cell cycle and differentiate, distinct neuronal subtypes emerge from progenitor cells in a highly controlled spatial order, partitioning the dorso-ventral axis of the neural tube into discrete regions. The correct coordination of these events requires tight spatio-temporal control. Sox genes could be at the core of that control as they play crucial roles during neural development. We have previously determined that Sox5 control cell cycle exit of neural progenitors through the inhibition of the mitogenic Wnt signaling (Martínez-Morales *et al.*, 2010). More recently, using gain- and loss-of function approaches, we have determined that Sox5 controls cell fate specification of dorsal neural progenitors inducing the transcription of the Wnt pathway negative regulator Axin2. We have established that Sox5 cooperates with βcatenin in the transcriptional activation of *Axin2*, through direct binding to *Axin2* enhancer regions. Thus, Sox5 can restrict proliferation and prevent the extent of dorsal identity, both imposed by Wnt signaling in the central nervous system.

P08 – Free Radicals and Oxidative Stress

P08-1
Oxidative modification of proteins in white rats blood under the acute acoustic stress conditions and α2–adrenoblocker administration

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On the basis of the existing data and results of our investigations we supposed that in the mechanisms of noise destructive action the leading role belongs to the significant disorders in pro-antioxidant activities of tissues, particularly activation of lipid peroxidation due to free radical oxidation intensification. The recent studies show that oxygen metabolism products also damage proteins. The goal of this investigation is to study the intensity of proteins oxidative modification (POM) in serum of experimental animals under conditions of acute acoustic stress using two different methods as well as effects of α_2–adrenoblocker administration on the studied parameters. The experimental animals (white male rats) underwent of 91 dBA noise level during 2 hours (acute acoustic stress). The part of animals was administered intraperitoneally α_2–adrenoblocker Beditine (synthesized in RA) in the dose 2 mg/kg. The first POM evaluation method is based on spectrophotometric determination of dinitrophenylhydrasones, which are formed with the carbonyl groups of aminoresidues of proteins. A marked increase in the level of dinitrophenylhydrasons in blood serum is an evidence of elevated protein oxidation under condition of acute acoustic stress. Beditine preliminary administration in a significant degree The study of the prevents increase of dinitrophenylhydrasones content. Bityrosine, the other marker of protein destruction, which is a product of phenylalanine and tryptophan aminoacids oxidation, under the same conditions revealed not statistically verified increase of bityrosine in all the sera samples. The data obtained are the evidence of serum proteins oxidation under the acute acoustic stress conditions which was more expressed according to the carbonyl groups content compare with bityrosine. All the above mentioned enables us to confirm the necessity to determine proteins oxidation products, i.e. carbonyl compounds as the free radical oxidation intensity markers under the acoustic stress condition.

P08-2
A closer look at the biosynthetic pathway of ergothioneine in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, despite intensive efforts, is still a major health concern causing close to 2 million deaths annually. Mtb's success, in part, is due to its ability to resist killing by reactive oxygen and nitrogen species encountered inside the macrophage. To protect from oxidative stress, cells normally use low molecular weight thiols. Ergothioneine (ESH) is an unusual sulfur-containing compound derived from histidine that is synthesized by certain bacteria and fungi. In mammals, ESH is obtained solely from dietary sources and has been detected in cells exposed to high levels of oxidative stress. The antioxidant cytoprotective role of ESH in mammalian physiology is becoming increasingly evident; however, its function in microbial cells remains unknown. According to previous findings, ESH synthesis enzymes may represent an attractive drug target in Mtb, thus further exploration of the thiol's role in the Mtb physiology is of interest. Based on *in silico* approaches, we have identified the gene cluster responsible for the production of ESH in Mtb. In this cluster, the protein encoded by *Rv3701c* was found to catalyze the trimethylation of histidine to form the pathway's first intermediate, hercynine. Further characterization of the enzyme (EgtD) revealed substrate specificity to histidine as no methylation activity was observed with any of the tested compounds. Lastly, *in vitro* phosphorylation of EgtD by Mtb's protein kinases was explored to determine if there is potential adaptive regulation of its activity in response to environmental stressors. These findings have provided us with a greater understanding of the biochemistry of ESH synthesis and will enable us to study its role in Mtb physiology more readily.

P08-3
Free radical oxidation disturbance in the rotenone animal model of Parkinson disease

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We investigated the balance of free radicals in brain different fractions (striatum, brain stem, neocortex, cerebellum & spinal chord) and some tissues (liver, heart, timus) of rats in course of *in vivo* processing by *rotenone*, a clinically-related animal model of Parkinson's disease (PD). Chemiluminescence (ChL) levels were examine in tissue assays on the 5th, 10th and 15th days after stereotaxical (ST) infusion of small doses of the mitochondrial complex-I inhibitor, rotenone, into the right medial forebrain bandle area. The TBA-test was also performed to confirm the free radical expression. The activity of superoxide dismutase in isolated tissue fractions were detected by spectrophotometry. Five days after ST rotenone administration, chemiluminescence levels of tissue homogenates significantly decreased in all fractions exept of striatum, while in 10th and moreover in 15th days of rotenone intoxication the level of ChL were elevated; lipid peroxidation also decreased in all fractions at 5th and 10th days, but there was no significant balance changes in the 15th day of treatment. On the contrary, the activity of superoxide dismutase was not shown any tendencies to change in all tissues, except of the neocortex and liver. In the latter's we observed the dramatically increased activity of the enzyme at the 10th day of rotenone injections.

P08-4
Resilience of molecular stress-related response in fish *Carassius auratus gibelio* following the history of population

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In the feral animals, combination of site-related environmental peculiarities and novel toxic effects could distort or even exceed the resilience of adaptive response. The aim of this study was to elucidate the contribution of the history of populations in the biological response of male fish *Carassius auratus gibelio* on the

effect of typical toxicants. Fish from reference and agricultural polluted sites were exposed to waterborne Cu, Mn, thiocarbamate or tetrazine pesticides during fourteen days. The traits of oxidative stress response, geno-, cyto- and neurotoxicity, vitellogenin production, cytochrome P450-related activity (EROD) were evaluated. The analyses also included concentration of metal-keeping proteins metallothioneins (MT) determined from metals, thiols and immunoreactivity. Initial difference in the health status of the fish from two sites was shown due to the weakness of antioxidant defense (superoxide dismutase and catalase activities, redox index of glutathione, superoxide anion, protein carbonyls, and lipid peroxidation levels), imbalance of the concentrations of different forms of MTs, and low cholinesterase activity in fish from polluted site. Under all exposures, fish from agricultural site demonstrated up-regulation of antioxidant defense and biotransformation systems. However, unlike specimens from reference site, this fish was unable to maintain metal homeostasis with the participation of MTs. Hence, the fish from the polluted site was better adapted to the detoxification of pesticides and less tolerate to the effect of toxic metals than fish from reference site. Among a set of biochemical markers, the characteristics of MTs and DNA abnormalities were selected by discriminant analysis as main distinguishing indexes of sites and exposures.

P08-5
Detection of nitrated fatty acids in olive oils: endogenous formation versus acidic nitration

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Nitric oxide (NO)-derived species react with unsaturated fatty acids yielding nitro-fatty acids (NFA), novel signaling mediators of cell function. Extra virgin olive oil (EVOO) is the main source of lipids in the Mediterranean diet and is associated with cardiovascular protection and lower mortality. Conjugated linoleic acid (CLA), present in vegetable- and animal- derived lipids, exerts antioxidant, anti-atherosclerotic and anti-inflammatory actions. Taking into account that NO can be formed in plants, the relative abundance of unsaturated fatty acids in EVOO and the anti-inflammatory actions of NFA, we are interested in determining the presence of NFA derivatives in EVOO and the nitration of fatty acids during digestion conditions. Our study concentrates on the analysis and detection of NFA by LC-MS/MS in three different EVOO from Jaen-Spain. After incubation with pancreatic lipase, nitrated-CLA (conj-LNO_2)-but not nitrooleic nor nitrolinoleic acids- was observed in the three oils tested; however, the levels of conj-LNO_2 were below the limit of quantitation of the method. As NFA are electrophilic, the presence of conj-LNO_2 was confirmed by looking for the covalent adduct with BME. The presence of the different non-modified CLA isomers in the oils was also confirmed. The formation of conj-LNO_2 increased under gastric mimic acidic conditions; the main conj-LNO_2 isomers formed were the *c*-9, *t*-11,12-conjLNO_2, *c*-9, *t*-11, 9-conjLNO_2 and the *t*-10, *c*-12, conjLNO_2. The biological actions of nitrated components of oils were analyzed in activated macrophages. Nitrated EVOO increased the expression of hemoxygenase-1 and decreased the expression of the inducible isoform of nitric oxide synthase, overall showing protective anti-inflammatory effects.

P08-6
Association between nitric oxide synthase 3 G894T and intron 4 VNTR genetic polymorphisms and ischemic stroke risk in Turkish population

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Nitric oxide synthase (NOS) catalyzes the generation of nitric oxide (NO), a primary vasodilator and regulator of blood flow and vascular tone. Because of these characteristics, varieties in the *NOS3* gene have been considered to contribute to the development of ischemic stroke. In this study, we aimed to investigate the possible association between NOS3 G894T and intron 4 VNTR polymorphisms and the risk of ischemic stroke on 245 patients and 145 controls by PCR-RFLP and allele specific PCR techniques, respectively. For the G894T polymorphism, while 33.5% patients and 37.9% controls had 894GG wild type, 2.8% patients and 6.9% controls had 894TT homozygous mutated genotype. The percentage of 894GT heterozygous genotype was 63.7% and 55.2% in patients and controls, respectively. Both G (0.653 versus 0.655) and T (0.347 versus 0.345) allele frequencies were similar between patients and controls. For VNTR polymorphism, there were 2 homozygous aa genotype, 71 heterozygous ab genotype and 172 homozygous bb genotype in patients. There were no significant difference between patient and control group with respect to a and b allele frequencies. Therefore, the results showed that NOS3 G894T and intron 4 VNTR polymorphisms may not play an important role in the etiology of the development of the ischemic stroke. However, when these polymorphisms were analyzed in hypertensive and diabetic subgroups, NOS3 G894T wild type was found as significant risk factor both in hypertensive (OR = 2.193, p = 0.026) and diabetic (OR = 2.607, p = 0.018) subjects. In addition, while in hypertensive group, NOS3 VNTR bb genotype was found to increase the stroke risk 4-fold, in diabetic group, it increased the stroke risk 3-fold, significantly.

Keywords: NOS3, G894T, polymorphism, intron 4 VNTR, ischemic stroke

P08-7
Altered fibrin susceptibility to plasmin-induced lysis in coronary artery disease patients: relationships with oxidative stress

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Fibrinogen, the main protein component of blood clot, is considered a risk factor for coronary artery disease (CAD). This study was performed to evaluate fibrin susceptibility to plasmin-induced lysis and the contribution of redox status to this process. From plasma of CAD patients (n = 30) and age-matched controls (n = 28) fibrinogen was purified. Fibrin clots were prepared using thrombin and then digested with plasmin. Aliquots from each digest (equivalent to 10 μg of fibrin) were loaded onto 4–12% Bis-Tris gels. After electrophoresis, gels were stained with

Coomassie blue and band intensities of stained gels were quantified by densitometry. Plasma redox status (protein carbonyls-PC, Thiobarbituric Acid Reactive Substances- TBARS, total antioxidant capacity -TAC) and fibrinogen carbonyl content were estimated. PC and TBARS in CAD patients were found to be significantly increased (3.03 ± 1 prot versus 1.01 ± 0.51 nmol/mg and 30.29 ± 11.1 versus 11.22 ± 3.3 pmol/mg, respectively) and TAC significantly reduced (301.24 ± 97.4 versus 470.18 ± 45.27 nmol/ml trolox eq.) compared to controls. Good and poor fibrin degrading patients were defined those with residual fibrin after plasmin digestion within or over the range of control subjects, respectively (26.4 ± 7.25% versus 60.35 ± 13.66%). Poor degrading CAD patients showed markedly increased PC and TBARS levels and reduced TAC, whereas in good degrading patients differences versus controls were less marked. Purified fibrinogen from the poor degrading CAD showed an increased extent of carbonylation (4.5-fold increase versus controls). Interestingly, plasma oxidative stress levels and fibrin oxidation were strongly and positively correlated to fibrin resistance to lysis. In conclusion, our results suggest that fibrin is the target of oxidative attack by ROS. These modifications can be associated with an increased atherothrombotic risk.

P08-8
Relationship between autophagy and ubiquitin-proteasome system during neuronal cell death under oxidative strees

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Oxidative stress leads to neuronal cell death by a variety of mechanistic routes. Autophagic cell death (ACD) occurs as a secondary, caspase-independent, programmed cell death (PCD) pathway. Overstimulation of autophagic activity is thought to cause ACD. Under conditions where the ubiquitin-proteasome system (UPS), another protein degradation pathway, is rendered inoperable, activation of compensatory autophagy may ameliorate the accumulation of damaged proteins. Here we evaluated cross-talk between UPS and ACD under oxidative stress. Primary murine cortical neurons were exposed to oxidative stress insults: hydrogen peroxide or SIN-1, a peroxynitrite generator. Using immunocytochemistry/confocal microscopy and western immunoblotting, neurons were monitored after exposure to the UPS inhibitor MG132 and autophagy inhibitors 3-methyladenine or bafilomycin A1 (the latter applied to authenticate activation of autophagy). Labeling patterns of western immunoblots for ubiquitinated proteins revealed increased UPS activity with oxidative stress, while addition of MG132 blocked clearance of ubiquitinated proteins. Further analyses focused on p62 (that regulates delivery of substrates to UPS and associates with lysosomal vesicles) and LC3 (an autophagy marker). Oxidative stress over 8 hour caused increased p62 expression and LC3-II conversion, which were both accentuated by MG132. This suggests that increased autophagic activity follows inhibition of UPS. Oxidative stress significantly increased intracellular puncta of p62 and LC3, monitored by immunocytochemistry. Co-localization of p62 and LC3 in a time-dependent manner in MG132-treated neurons implicates p62 in ACD. Current studies focus on p62 to determine if this protein is the switch between cytoprotective autophagy and ACD.

P08-9
Basal, H_2O_2-induced and post-repair DNA damage, total antioxidant capacity and urinary 8-hydroxydeoxyguanosine level in childhood lymphoblastic leukemia survivors

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Chemotherapy and radiotherapy cause genetic damage in healthy cells. Most important side effect of anti-cancer therapy is development of secondary malignancy in cancer survivors. Oxidative DNA damage and DNA repair capacity may be determinative for secondary malignancy in cancer survivors. 8-hydroxydeoxyguanosine (8-OHdG) is the most mutagenic and abundant lesion caused by oxidative stress and measured as a marker of oxidatively damaged DNA. Xeroderma Pigmentosum group D (XPD) and X-ray repair cross-comlementing group 1 (XRCC1) are the DNA repair proteins involved by base excision repair system. In the present study, basal, H_2O_2-induced and post-repair DNA damage, urinary 8-OHdG level and serum total antioxidant capacity were measured in childhood acute lymphoblastic leukemia (ALL) survivors who had no leukemia symptoms at least one year. Polymorphisms at XPD 751, XRCC1 399 and XRCC1 194 had been analyzed previously in all cases. Basal, H_2O_2-induced and post-repair DNA damage in peripheral leukocytes were determined by the Comet assay. Serum total antioxidant capacity and urinary 8-OHdG level were measured by spectrophotometric and ELISA kits, respectively. Basal and induced DNA damage were found to be higher in ALL group versus control group. No significant difference was determined between groups for post-repair DNA damage, urinary 8-OHdG level and serum total antioxidant capacity. There was no relation between DNA repair capacity and polymorphisms of XPD 751 and XRCC1 399. However, XRCC1 194 Trp allele was found to be associated with high DNA repair in ALL survivors. In conclusion, basal DNA damage and susceptibility to oxidation is high in childhood ALL survivors but this situation is not derived from deficient DNA repair.

P08-10
The effect of caffeine on AOPP and MDA levels, superoxide dismutase and glutathione *S* transferase activities of rat kidney tissue

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Nowadays, caffeine is a substance that is consumed regularly everyday. Coffee, tea, chocolate, coke, energy drinks, diet pills, many drugs such as some analgesics contain caffeine. Today, it became the topic of many researchs because of its effects on tissue metabolism and antioxidant property. In our research, we investigated the effects of caffeine on advanced oxidation protein products (AOPP) and Malondialdehyde (MDA) levels, superoxide dismutase and glutathione s transferase activities of rat kidney tissue. In the study, 30 male wistar rats are used and divided into three groups randomly: control group and two caffeine groups. Caffeine is administered orally for 14 days to Group I at a dose of

30 mg/kg and Group II at a dose of 100 mg/kg. Our results show that 14-day low dose (30 mg/kg) and non-toxic dose (100 mg/kg) caffeine usage decreased protein oxidation in kidney. AOPP levels of kidney tissue decreased significantly compared to control group; however, this decrease is found to be independent from dose. The most decrease is found in Group II. Tissue MDA levels are found to be decreased with caffeine. A statistically significant difference is found between control group and caffeine groups. The most decrease occured in Group II. This antioxidant effect of caffeine is interpreted to be closely related with dose. GST activities in rat kidney showed statistically significant increase with caffeine intake; however, this increase is found to be independent from dose. The most decrease is found in Group II. In SOD activities of kidney, there was no statistically significant difference among the groups. These results support protective effects of caffeine from oxidative stress in short term different doses consumption.

P08-11
Oncogene driven redox cell survival mechanisms

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Reactive oxygen species (ROS) are a group of molecules produced in the cell through metabolism of oxygen. It is now evident that at low physiological levels, hydrogen peroxidefor examplecan act as a classical intracellular signalling molecule regulating phosphatase/kinase driven cell signalling pathways. The oncogenes Bcr-Abl and Flt-3 are central to the development of both chronic and acute leukaemias and when these two genes are expressed on a tet regulated expression system they are associated with increased ROS levels. Using confocal microscopy we have demonstrated that the Flt-3 and Bcr-Abl driven ROS production, in particular hydrogen peroxide, contributes directly to increased genomic instability seen in these tumour cells. We also show by western blotting that there is an increased flux through the Pi3K/Akt survival pathway. Using siRNA and pharmacological inhibitors we demonstrate that the source of the ROS is NADPH Oxidase. Inhibition of this enzyme system lowers ROS levels and also the ability of the tumour cell to survive. Using multi-photon microscopy and hydrogen peroxide specific dyes we demonstrate that the ER is the site of this ROS production. Direct pharmacological inhibition of clinically used Bcr-ABl and Flt-3 inhibitors is associated with a marked reduction in ROS production and we show this is due to ubiquitination and proteasomal destruction of p22phox which is a component of the NADPH Oxidase enzyme system. This in turn facilitates the cell death inducing properties of these chemotherapeutic agents by removing the ROS element of oncogene survival signalling. Thus the tumour survival promoting properties of the Bcr-Abl and Flt-3 oncogenes are driven in part through their ability to stimulate the production of ROS.

P08-12
Effects of some pesticides on catalase and glutation *S*-transferase in Cyprinus carpio carpio

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Free radicals which known reactive oxygen species (ROS) have changing capasity biomolecules like lipids, nucleic acids, considerably reactive and unstabile metabolites. Biological sistems in body developed defence system because of prevent the effect of ROS. This system known 'antioxidant defence systems' or shortly 'antioxidans'. Pesticides may induce oxidative stress, leading to generation of free radicals and cause lipid peroxidation. Due to using of pesticide in agriculture, which fishes are living in polluted freshwaters effect of negative direction. In this study, we investigated some pesticides effects on catalase (CAT) and glutation *S*-transferase (GST) enzymes obtain from Cyprinus carpio carpio. For this study, today using at agriculture of five different pesticides(2,4- dichloroplenoxy acetic acid dimethylamine salt, fenpropathrin, cypermethrin, lambda-cyhalothrin, deltamethrin) was elected. The inhibitory effect of these pesticides on catalase and glutation s-transferase activity, observed under *in vitro* experimental conditions. The pesticides used in this study inhibited the catalase and glutation *S*-transferase activity from Cyprinus carpio carpio to various degrees. These findings observed *in vitro* could be useful in the understanding of the toxic effects that pesticides elicit on aquatic organisms *in vivo*.

P08-13
The effect of sodium tetraborate on antioxidant enzymes

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Antioxidant enzymes have main role in the defense of mammalian blood. Experimental investigations have repeatedly shown that erythrocytes are particularly sensitive to oxidative stres. For this purpose, the *in vitro* biochemical effects of sodium tetraborat on the human blood was investigated in this study. We observed enzyme activities of erythrocyte superoxide dismutase, catalase, glutathione reductase, glutathione s-transferase, glutathione peroxidase, glucose-6-phosphate dehydrogenase and also the effect of sodium tetraborat on antioxidant enzymes by spectrophotometrically. All the heparinized blood samples were taken from 10 healthy people between 25 and 35 who were not exposed to any toxic agents and did not smoke or drink. In this study blood samples were exposed to various doses (2, 4, 8, 17, 33 ppm) of sodium tetraborat *in vitro* conditions. In conclusion, the results suggested sodium tetraborat had shown neither inhibition nor activation effect on antioxidant enzyme activities.

P08r-14
Friedreich Ataxia: rat ventricular myocytes deficient in frataxin have disrupted mitochondria and impaired metabolism

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Friedreich ataxia (FA) is an inherited neurodegenerative disorder characterized by progressive gait and limb ataxia. Other variable features of FA disease include visual defects, scoliosis, and cardiomyopathy. It occurs in about 1 in 50 000 Caucasians. It is caused by mutations in the gene encoding frataxin, a mitochondrial protein which is depleted in FA. Although the exact function of frataxin is still controversial, the current commonly accepted hypothesis confers a role for frataxin in iron metabolism and in oxidative stress protection within the mitochondria. An important number of FA patients suffer from diabetes and most of them have evidence of cardiac dysfunction in live. Even though heart failure is the most frequent cause of death in these patients, the effects of frataxin depletion on cardiomyocytes are poorly understood. Disarrangements in the cardiac function could be caused by alterations in heart myocytes, which are rich

in mitochondria and present high oxidative metabolic rates. In this context, we have developed a cardiac model of frataxin deficiency using primary cultures of rat neonatal ventricular myocytes, which have been transduced with lentivirus containing shRNA against frataxin mRNA. We have analyzed the metabolism and mitochondria of these cells, together with markers of oxidative stress. Our results indicate that lack of frataxin triggers oxidative stress, metabolic rearrangements and mitochondria disarrangements, which could be the origin of the altered cardiac status found in most FRDA patients.

P08r-15
Cyanobacterial FurA protects from oxidative stress presumably by a disulfide bond-mediated mechanism

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Ferric uptake regulator (Fur) proteins constitute a family of prokaryotic transcriptional regulators with a wide diversity of functions. FurA is a global transcriptional regulator in the filamentous, nitrogen-fixing cyanobacterium *Anabaena* sp. PCC7120. It is an essential protein that links iron metabolism to the nitrogen fixation. FurA also participates in the oxidative stress response, among other processes (1). This DNA-binding protein contains five conserved cysteine residues, whose redox status is a key issue to maintain FurA in an active state (2). Four cysteine residues are included into two redox CXXC motifs ($C_{101}VKC_{104}$ and $C_{141}PKC_{144}$), while the fifth one (C_{133}) is specially conserved among the FurA orthologs identified in cyanobacteria. Electrophoresis studies and crosslinking assays were performed with multiple cysteine FurA mutants to analyze protein oligomerization *in vitro* mediated by inter and intramolecular disulfide bonds. Overexpression of FurA from *Anabaena* protected *Escherichia coli* from oxidative damage *in vivo*, presumably by establishing disulfide bonds leading to FurA oligomerization. Our data also revealed that *E. coli* strains overexpressing triple cysteine FurA mutants, which presented lower amount of oligomeric species *in vitro*, were more sensitive to oxidative damage caused by H_2O_2 *in vivo*. We propose a new mechanism of action of FurA inside the cell, besides its role in transcriptional regulation. Under oxidative stress conditions, FurA could act as a reactive oxygen species scavenger by establishing disulfide bridges between its cysteine residues. Overall, the present study confirms the importance of disulfide bond-mediated oligomerization of FurA *in vivo* to increase cell survival under oxidative stress conditions.

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P08-16
Protective effect of xanthohumol on acute alcohol-induced injury in rat liver and kidney

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Reactive oxygen species (ROS) are implicated in a variety of human diseases, however these species are scavenged by antioxidant defense system that include enzymes as catalase and SOD, and antioxidants such glutathione. Metabolism of ethanol gives rise to ROS, mainly in liver, where is extensively metabolized to acetaldehyde by alcohol dehydrogenase. In this investigation we evaluated the oxidative stress produced by ethanol in liver and kidney and the protective effect of a pre-treatment with xanthohumol (XN), a prenylflavonoid present in hops plant that exert anti-oxidant, anti-inflammatory and anti-angiogenic properties. This compound is one of the major flavonoids present in beer. For this study, Wistar rats were treated with ethanol (4.5 mg/kg bw) and the effect on antioxidant enzymes catalase and SOD were evaluated. Also, lipid peroxidation, GSH and H_2O_2 were measured to know the level of oxidative stress induced by ethanol treatment. Administration of ethanol caused significantly enhanced lipid peroxidation and H_2O_2 concentration, with marked depletion of GSH. Concomitantly, a reduction of catalase and SOD activities was produced. Our results shows a maximum level of stress oxidative in liver and kidney 12 hour after ethanol administration, being more intense the oxidative damage in liver than in kidney. When ethanol-intoxicated rats were pre-treated with XN at doses of 0.1, 0.2 or 0.4 mg/kg bw, a marked and dose-dependent protective effect against alcohol-induced toxicity was observed. XN significantly lowered lipid peroxidation (64%) and hydrogen peroxide concentration (88%) in ethanol treated rats. Also, GSH concentration and enzymatic activities of catalase and SOD in ethanol-treated rats were increased by a 92%, 73% and 64% respectively, when pre-treated with XN, reaching values near to the obtained for rats in absence of ethanol treatment. The results obtained demonstrate that XN produces a beneficial effect in reducing the oxidative damage of ethanol on liver and kidney. This work was supported by grant GR10139 from the Junta de Extremadura (FEDER funds).

P08-17
Xanthohumol prevents carbon tetrachloride-induced acute liver injury in rats

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Xanthohumol (XN), a prenyl flavonoid present in beer, prevents the acute hepatic injury induced by carbon tetrachloride (CCl_4) in rats. Pre-treatment of rats with XN significantly reduced the increased liver weight observed in CCl_4-intoxicated rats, normalised the increased values of plasma lactate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities and reduced the incidence of histopathological alterations produced by CCl_4. The oxidative stress induced by CCl_4 administration elicited a significant decrease in the levels of reduced glutathione as well as an increase in thiobarbituric acid reactive substances (TBARS) and H_2O_2 concentrations. Pre-treatment of rats with XN resulted in a significant ($p < 0.05$) increase in reduced glutathione (GSH) content and a reduction in TBARS and H_2O_2 concentrations to their normal values. XN pre-treatment also prevented the significant reductions of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase activities observed in CCl_4-treated rats compared to control animals. Our results suggest that the hepatoprotective effect of XN is based on its antioxidant properties as well as it being an efficient inhibitor of lipid peroxidation and a protector against the degradation of antioxidant enzymes induced by CCl_4 intoxication. This work was supported by grant GR10139 from the Junta de Extremadura (FEDER funds).

P08-18
The ERα/ERβ ratio determines oxidative status in postmenopausal women mammary carcinomas

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Breast cancer is one of the most common female malignancies and occurs more frequently among postmenopausal women in comparison to premenopausal women, it is known to be an estrogen-dependent malignancy. There are two different subtypes of estrogen receptors (ERα and ERβ), which present different distribution and functions. ERα plays an important proliferative role, increasing tumor growth and oxidative stress. However, ERβ appears to reduce cell proliferation induced by ERα and promote differentiation in the mammary gland. The aim of this study was compare oxidative stress in different breast tumors according to ERα/ERβ ratio, given that ERs regulate several proteins related to oxidative status. Tissue samples were obtained from 13 postmenopausal women between 50 and 70 years old, who presented an Invasive Ductal Carcinoma (IDC). All tumors were characterized and classified according to ERα/ERβ ratio, determined by Western blot. Oxidative damage was measured. Antioxidant enzymes, uncoupling proteins (UCPs), Sirtuin3 and mitochondrial respiratory chain complexes were determined by Western blot. Moreover, AKT and SAPK/JNK protein levels and phosphorylation state were also studied. Results showed that tumors with low ERα/ERβ ratio have great oxidative damage, more activation of proliferation-related and oxidative stress-activated signaling pathways and a decrease in OXPHOS protein levels, indicative of mitochondrial dysfunction. These tumors exhibited an up-regulation of antioxidant enzymes as well as UCP5 and Sirtuin3 protein levels. On the whole, these results show that tumors with low ERα/ERβ ratio have poor prognosis suggesting that it would allow them to better adapt to a situation of oxidative stress, thus favoring their survival.

P08-19
Superoxide dismutase isoenzymes as markers for yeast strains differentiation by zymogram analysis

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Metabolic activities inside the cell produce oxidative stress by the generation of reactive oxidative species (ROS) (1). Aerobic organisms have devised several enzymatic and non-enzymatic antioxidant defenses to deal with this oxidative stress. One of the mechanisms to combat such stresses involves induction of free radical scavenging enzymes such as catalase, peroxidase, superoxide dismutase (SOD) and glutathione reductase. Isoenzymes of SOD (Sod1p and Sod2p) and catalase (cta1p and ctt1p) have been reported in the yeast *Saccharomyces cerevisiae* but there is little knowledge about them in other yeasts. Recently, a technique for double staining of SOD and catalase in the same polyacrilamide gel has been described (2). In the present investigation, we have improved and developed this technique for other *Saccharomyces* species and also other non-*Saccharomyces* yeast strains finding unique zymographic patterns of SOD which can be used to distinguish between these organisms. Moreover, we have developed a new technique to detect differentally Sod1p and Sod2p on the gel by immersing it in NaCN, which inhibits specifically the Sod1p isoform. Therefore, we propose the use of the zymographic patterns of SOD for classification of *Saccharomyces* and non-*Saccharomyces* yeast strains.

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P08-20
Antioxidative effects of novel selenium compounds

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We have shown an antioxidant and proliferative influence of novel selenium compounds of 2- (phenylselenomethyl) tetrahydrofuran and 2- (phenylselenomethyl) tetrahydropyran in concentrations from 0.1 to 500 μM for 24 hour and 72 hour from treatment. Investigated model systems were breast cancer cell line MDA-MB-231 and colon cancer cell line HCT-116. We monitored cell viability in a concentration-dependent manner by using an MTT assay. Both compounds exhibited no cytotoxic effect and had enhanced proliferation effect, with IC_{50} values greater than 500 μM. Among the oxygen radicals, we measured the concentration of superoxide anion radical ($O_2^{\bullet-}$) and it was shown spectrophotometricaly that MDA-MB-231 cells exhibit decreasing of $O_2^{\bullet-}$ as well as HCT-116 cells after treatment with both selenium compounds. Also spectrophotometric determination of nitrites – NO_2^- (indicator of the nitric oxide-NO level) was performed by using the Griess method. MDA-MB-231 cells and HCT-116 cells exhibit increasing of nitrite concentration after treatment with selenium compounds with slightly greater increasing of nitrite level after 72 hour compared to 24 hour. We performed assay for spectrophotometricaly quantitative determination of glutathione. We monitored that increased glutathione content and increased concentration of selenium compounds synergicaly affected the superoxide anion radical production. Our findings showed that investigated selenium compounds play an important role in reducing the levels of reactive oxygen species; therefore, we believe that as antioxidants they could serve in preventing the processes arising as a consequence of oxidative stress, including cancer.

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P08-21
Brain mitochondria and long-lasting psycho-emotional stress

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Against the background of stress induced by 30-day isolation and disruption of circadian rhythms, we have studied biochemical changes occurring in brain cell mitochondria of white rats. It turned out that psycho-emotional stress is a factor causing a number of complex changes in brain cell mitochondria. Such change includes a pathological process consisting in development of oxidative stress, indicated by a dynamics of accumulation of lipid peroxidation products in mitochondria and decreasing enzyme activity in the anti-oxidant system as a result of 30-day isolation and violation of circadian cycle among animals.

We have also discovered that development of oxidative stress is accompanied by a decreasing energy potential of brain cells, indicated by a reduction in the activity of the aerobic and anaerobic enzymes involved in energy metabolism. Another signal of the process is a sharp decrease in the activity of creatine kinase in brain mitochondria.

Along with a reduction in creatine kinase activity, the quantity of enzyme substrate creatine has also been found to decrease when animals are subjected to 30-day isolation and violation of circadian cycle.

Creatine is known to have a protecting role in defining functional status of mitochondrial permeability transition pore (MPTP). It has been discovered that the stress caused by 30-day isolation and violation of circadian cycle is a factor causing swelling of rat brain mitochondria and hence, opening of the mega-channel, in its turn leading to apoptosis of cells, death of neurocells and a number of resulting pathologies.

P08-22
Luminal H_2O_2 production promotes, while elimination impairs *in vivo* disulfide formation in endoplasmic reticulum

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Oxidative protein folding in the luminal compartment of endoplasmic reticulum (ER) is thought to be accompanied by the generation of H_2O_2, as a side-product of disulfide bond formation. We aimed to examine the role of H_2O_2 produced in the lumen, which on one hand can lead to redox imbalance and hence contribute to ER stress; on the other hand, as an excellent electron acceptor, H_2O_2 might serve as an additional prooxidant in physiological oxidative folding. Elevation of hepatic luminal H_2O_2 levels of mice resulted in a decrease in thiol content and in a redox shift of certain luminal oxidoreductases towards oxidized direction. The oxidative wave was accompanied by moderate signs of ER stress and reversible dilatation of ER cisternae, all effects prevented by concomitant reducing treatment. The imbalance also affected the redox state of glutathione; the GSH/GSSG pool was severely decreased in mice where H_2O_2 was overproduced. Antibody producing cells artificially engineered with powerful luminal H_2O_2 eliminating system showed diminished secretion of mature antibody polymers, while incomplete antibody monomers/dimers were secreted and/or accumulated. Evidence were firstly provided by using *in vivo* models that H_2O_2, instead of being a harmful byproduct of oxidative protein folding, can promote disulfide bond formation in the ER.

P08r-23
Folate deficiency causes premature hearing loss in C57BL/6J mice

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Both environmental and genetic factors contribute to hearing loss. Alterations in plasma homocysteine (pHcy) levels and betaine homocysteine methyltransferase (BHMT) expression have been reported in genetic mouse models of human deafness (1). Dietary and nutritional factors can also modulate Hcy metabolism. BHMT is one of the enzymes responsible for Hcy remethylation leading to methionine synthesis, its changes being a direct cause of increased pHcy levels and indirectly altering production of the antioxidant glutathione (2).

Here we have studied the impact of a dietary induced folic acid deficiency on the cochlear methionine metabolism and in hearing. Hearing capacity was assessed by ABR threshold analyses after 4 weeks of treatment with either the control or the folate deficient diets. Blood and tissue samples were used to obtain pHcy concentrations by HPLC and the levels of methionine metabolism enzymes by western blot. Cochlear morphology was evaluated by hematoxylin and eosin staining and immunohistochemistry techniques. The expression of the corresponding mRNAs was also evaluated using real-time PCR.

The results obtained indicate that folate deficiency causes alterations in the cochlear methionine cycle leading to an increase in pHcy levels, which is concomitant with molecular and cellular alterations in this organ and premature hearing loss in the C57BL/6J mouse.

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P08-24
Effect of oxidative stress on HNE metabolism

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4-Hydroxy-2,3-nonenal (HNE) is a major aldehydic end product of lipid peroxidation. Due to its high reactivity, it has various cytotoxic, mutagenic and genotoxic effects on mammalian cells. Experimental evidence suggests that the intracellular concentration of HNE may differentially affect the cell cycle regulation. It has been shown that at low concentration, HNE causes proliferation, but at higher level it causes differentiation and apoptosis. In

recent years main attention was given to cellular detoxification of HNE and to the possible role of the aldehyde as signaling molecule during oxidative stress in association with important diseases such atherosclerosis and cancer. The enzymatic activity and protein expression of HNE metabolizing enzymes (i.e. aldose reductase, glutathione *S*-transferase and aldehyde dehydrogenase) were monitored in different cell lines (bovine lens epithelial cells and human astrocytoma ADF cells) undergoing oxidative stress. A different degree of severity of oxidative stress was imposed by incubating the cells in the presence of different concentrations of hydrogen peroxide. At the end of incubation cell viability, glutathione content and HNE metabolizing enzymes activities were evaluated. While no changes in aldose reductase and glutathione *S*-transferase occurred, a significant reduction of aldehyde dehydrogenase activity was detected in bovine lens epithelial and ADF cells. The significance of channelling HNE into the reductive pathway in conditions of oxidative stress and its relevance for the red/ox signalling machinery are under investigation.

P08-25
Immunosuppressive drugs interaction: cytotoxicity and oxidative damage in intestinal HCT 116 cells

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Immunosuppressive agents are drugs that inhibit or prevent activity of the immune system. They are used to treat the rejection of transplanted organs and autoimmune diseases. These treatments can cause toxic effects at very low doses that may modulate cellular response. The toxicity of immunosuppressant drugs varies with the duration of treatment and drug interactions in transplant. Among the immunosuppressant, Tacrolimus is a macrolide lactone which acts by inhibiting calcineurin. Therapeutically it is always associated with a second molecule, Mycophenolate Mofetil (MMF), which is a selective and reversible inhibitor of inosine monophosphate dehydrogenase (IMPH). MMF and Tacrolimus combination has become the most commonly treatment used after allograft. This study aimed to characterize the gastrotoxicity risk profile for combination therapy of Tacrolimus and MMF compared to their effect separately. We were interested to evaluate the cytotoxicity and oxidative cell damage *in vitro*. The effect of Tacrolimus and MMF used in combination or separately were assessed on cell proliferation using cell viability tests in intestinal HCT 116 cells. In addition, we determined the oxidative stress of the immunosuppressive drugs by measuring the level of free radicals and lipid peroxidation. Our data showed that Tacrolimus induced oxidative stress in HCT116 cells. The oxidative damage is evidenced by the increase of reactive oxygen species (ROS) and lipid peroxydation. Combination of Tacrolimus with MMF at low concentrations shows a preventive effect of oxidative stress induced by Tacrolimus. This preventive effect is transformed from a certain concentration of MMF in a synergistic toxic effect.

P08-26
Reactivity of sulfenic acid in human serum albumin

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Sulfenic acid is a central intermediate in the redox modulation of several proteins. However, information regarding its reactivity is scarce. We have characterized the sulfenic acid formed in human serum albumin (HSA-SOH) when its single thiol, Cys34, is exposed to oxidants. Once formed, HSA-SOH can have three possible fates. First, it can be further oxidized by hydrogen peroxide to sulfinic acid (HSA-SO_2H) with a rate constant of 0.4 M/s (pH 7.4, 37°C). The formation of HSA-SO_2H was confirmed by mass spectrometry with whole and trypsinized protein. Second, HSA-SOH can react with low molecular weight thiols forming mixed disulfides. Through a competition approach using the yellow thiol thionitrobenzoate, we determined the rate constants for the reactions between HSA-SOH and different thiols, concluding that the mechanism involves the nucleophilic attack of the thiolate. Thiols with positive net charge at pH 7.4 reacted faster than the negative ones. Third, HSA-SOH can decay spontaneously in solution (1.7×10^{-3} sec^{-1}, pH 7.4, 37°C). The product formed (HSA-SX) was analyzed by mass spectrometry of the whole protein, after ion exchange chromatography to separate the different thiol oxidation states. HSA-SX had a mass of 66437 ± 2 Da which, within experimental error, was the same mass as the thiol form of HSA. However, HSA-SX could not be alkylated by *N*-ethylmaleimide as occurs for HSA-SH. Treatment with 2-mercaptoethanol reversed this modification. Our results are consistent with the formation of a sulfenamide (HSA-S-NH-R), although the nature of the reacting amine or amide remains elusive. Finally, we are working on the production of native and mutant recombinant HSA to further characterize HSA-SOH.

P08-27
Evaluation of cytokines concentrations and oxidative DNA damage in patients with diabetic nephropathy and non- diabetic nephropathy undergoing hemodialysis

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Aim: We aimed to evaluate pro-inflammatory cytokines (TNF-alpha and IL-6) and 8-hydroxy-2′-deoxyguanosine as oxidative stress biomarker concentrations in patients with diabetic nephropathy and non-diabetic nephropathy undergoing hemodialysis
Methods: This study is conducted serum samples of 28 patients with diabetic nephropathy and 28 patients with non-diabetic nephropathy in pre- and post-hemodialysis. Values of IL-6, TNF-alpha and 8-OhdG were determined by ELISA kits in pre-and

post-hemodialysis. Additionally, these parameters compared to healthy control subjects.

Results: We found thatthe TNF-alpha values were higher in both pre- and post-hemodialysis in patients with diabetic nephropathy, when compared to the controls. And TNF-alpha values of patients with non-diabetic nephropathy were also higher in both pre- and post-hemodialysis in patients with non-diabetic nephropathy. Moreover, IL-6 values in post-hemodialysis were higher than pre-hemodialysis in patients with both diabetic nephropathy and non-diabetic nephropathy. Serum 8-OHdG levels were found increased in all patients groups in pre and post-hemodialysis when compared to controls.

Conclusion: According to oxidative DNA damage in patients with diabetic nephropathy treated with hemodialysis due to chronic renal failure was higher than patients with non-diabetic patients treated with hemodialysis due to chronic renal failure. In contrast, inflammation in patients with diabetic patients was found to be lower than in patients with non-diabetic nephropathy.

P08-28
Characterization of ascorbate peroxidase activity from the eukaryotic microalga *Coccomyxa acidophila*

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Hydrogen peroxide and other active oxygen species are produced as a result of many biochemical and physiological reactions and are considered to be the primary factors in oxidative damage. Ascorbate peroxidase (APX; EC 1.1.1.11) is a hemeprotein that plays a key role in scavenging excess hydrogen peroxide (H_2O_2), using ascorbate as electron donor. APX has been identified in many higher plants and constitutes a family of isoenzymes, with differences physico-chemical and kinetic properties, distributed in different subcellular compartments. In the present study, we have characterized the APX activity assay in crude extract of the eukaryotic microalga *Coccomyxa acidophila*, and also identified tow isoforms of the enzyme. The microalga is an extremophile organism isolated from the Tinto River mining area in Huelva (Spain), that can live at extremes pH (< 2.5), temperature, salinity and high concentration of heavy metals. Crude extracts from *C. acidophila* were obtained by sonication disruption, and streptomycin sulphate was required to eliminate nucleic acids and pigments. The enzyme showed an optimal pH of 7.0, in 50 mM phosphate buffer and an optimal temperature of 50°C. APX showed a high thermal stability from 0 to 30°C during 400 min. Kinetic characterization of APX, in crude extract of the microalga, led to obtain a V_{max} of 555 U·mg prot and a K_m value of 39 μM for ascorbate. Nevertheless, the study for H_2O_2 showed a sigmoidal kinetic, with a $S_{0.5}$ of 1.3 mM and a n_h of 2.3, which indicates an allosteric behaviour of the APX activity for this susbtrate. Inhibition studies let to a high inhibition of APX by DTT (1 mM) or cyanide (1 mM) and the enzyme showed an uncompetitive inhibition by Mn^{2+}. DEAE-sephacel chromatography of the crude extract from *C. acidophila* allowed the identification of two peaks with APX activity. These isoforms, named APX1 and APX2, showed different kinetic properties. Supported by research project no AGL2007-65303-C02-01

P08-29
Copper binding to S100A12: suggested role in oxidative modification of lipids and proteins

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S100A12 is a member of the S100 family of EF-hand calcium-binding proteins. Extracellular S100A12 is predominantly secreted by neutrophil granulocytes and is part of the innate immune response. S100A12 is markedly overexpressed in inflammatory compartments, and elevated serum levels of S100A12 are found in patients suffering from inflammatory and metabolic disorders, such as rheumatoid arthritis and type 2 diabetes mellitus. Besides calcium S100A12 also binds zinc and copper ions. In this regard, copper binding to S100A12 is hypothesized to influence the redox balance in (pro)inflammatory states. Both ultrafiltration assays and copper-64 radionuclide binding experiments *in vitro* show that one molecule dimeric recombinant human S100A12 binds 2 atoms of copper (Cu^{2+}/Cu^{+}) in His motifs with a dissociation constant of about 0.02 μM. Copper-bound S100A12 can function as a pro-oxidant agent by supporting both copper reduction and copper redox-cycling, respectively. As a consequence, copper-bound S100A12 (10 μM) enhances and accelerates oxidation of human LDL lipids (decrement in lag-time of LDL conjugated diene formation by $> 15\%$, $p < 0.01$) and apolipoproteins (increment in apoB-100 ©-glutamyl semialdehyde/α-aminoadipyl semialdehyde content by $> 30\%$, $p < 0.01$). These processes were substantially suppressed in the presence of redox-inert copper-chelating or radical-scavenging agents. Redox-properties of S100A12 are compared with that of other copper-binding S100 proteins, e.g., S100B. It is suggested that oxidation processes mediated by copper-bound S100A12 are involved in accelerated atherogenesis in inflammatory and metabolic disorders.

P08-30
Mutational and selective forces cooperate to shape sulphur-containing amino acids content in mammalian mtDNA-encoded proteins

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A number of studies have suggested that during the course of mammalian evolution, a decreased cysteine usage along a gain of methionine and threonine residues into mtDNA-encoded proteins, might have resulted in proteomes better endowed to resist oxidative stress. However, owing to the problem of distinguishing between functional constraints/adaptations in protein sequences and mutation-driven biases in the composition of these same sequences, the adaptive value of the above referred amino acid shifts has recently been put in doubt. Herein, we have addressed this issue by comparing the coding sequences of mitochondrial genomes from 173 mammalian species, trying to dissect the effect of nucleotide composition on the cysteine, methionine and threonine usages. We found that the nucleotide composition bias is the main, if not the only, driving force in threonine composition of the encoded proteins. In contrast, nucleotide composition has not effect at all on the cysteine usages, which seem to be kept at low values by purifying selection. With respect to methionine, the results suggest that both nucleotide bias and selective forces unrelated to the nucleotide composition, contribute to shape the methionine content in mtDNA-encoded proteins.

P08r-31
The Aldo-Keto Reductase (AKR) superfamily as an experimental model for the study of protein oxidation

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A common problem in the biotechnology industry is the oxidation of therapeutics proteins. The susceptibility to chemical oxidation of a particular residue depends on its chemical nature and its structural environment. That is, a given residue can exhibit a wide range of reactivities against oxidants depending on its structural context. However, these structural determinants remain largely unknown. The aim of the current work was to set an experimental model suitable to correlate the degree of resistance to oxidation of a protein with structural features of that protein. For this purpose, we focus our interest on the superfamily of AKR for several reasons. The AKRs are monomeric proteins that are present in most organisms. This superfamily encompasses more than hundred described members, which shares a high degree of sequence identity and the same (αβ)8-barrel motif. On the other hand, these enzymes act on a diverse range of substrate and are involved in many and very different cellular functions. Since a good heuristic indicator of a protein family's evolvability is its natural functional diversity, we aimed to find members showing a differential susceptibility to oxidants and correlate their vulnerability with structural parameters. To this end, we have cloned, expressed and purified a set of homologous AKR. The kinetic of inactivation, in presence of H_2O_2, of each homologous protein was recorded at different concentrations of the oxidant. We describe the existence of a differential susceptibility to oxidation between homologous members from the AKR superfamily, which seems to be related to structural determinants such as protein compactedness and the accessible surface area of specific residues.

P08-32
Study of the involvement of DJ-1 in autophagy. Importance of the Nrf2/Keap1 axis

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DJ-1 (CAP1/RS/PARK7) is a protein of 189 amino acids encoded by *PARK7* gene, which plays a central role in the cellular biology. Multiple studies have shown that homozygous loss-of-function mutations in DJ-1, result in early-onset Parkinson Disease (PD). Although function of DJ-1 is unclear, several studies suggest their possible involvement in the cellular response against oxidative stress, because DJ-1 has the capacity to eliminate reactivity oxygen species (ROS) by auto-oxidation. In basal conditions, DJ-1 is mostly in the cytosol and found in less quantity in nucleus and mitochondria. In oxidative stress conditions, DJ-1 is redirected to mitochondria and subsequently to the nucleus, in order to exert its antioxidant action. There are cellular and animal models to investigate the combined effect of genetic and environmental factors involved in PD and to study the interaction between DJ-1 and the herbicide paraquat (PQ). In this study, we observed differences in (ROS), cell sensitivity and protein levels of DJ-1 in murine embryonic fibroblasts (MEFs), which are genetically manipulated and it have reduced levels of Nrf2 and Keap1. So that, Nrf2-KO cells show increased levels of ROS, decreased viability and lower levels of DJ-1 with and without PQ treatment, if compared it with wild type or Keap1-KO. These results suggest a possible role of DJ-1 in the autophagy response against PQ and can reduces apoptotic cell death response. These findings could *be important* to the development of *new treatments strategies* against PD. The funding was from Junta de Extremadura (GR10054); FUNDESALUD (PRIS11014, PRIS11019), ISCIII, Ministerio de Economía y Competitividad (CB06/05/0041, PI11/0040). J.M.B-S was supported by a postdoctoral fellowship (Junta de Extremadura, Spain), R.G-S. was supported by a FPU predoctoral fellowship (Ministerio de Educación, Spain), M.N-S. was supported by a postdoctoral fellowship (Junta de Extremadura, Spain), Dr. R.A. G.-P. was supported by Miguel Servet contract, ISCIII (Ministerio de Economía y Competitividad, Spain).

P08-33
The potential role of combined antioxidants against indomethacin-induced liver injury in rats

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Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used medicines throughout the world. NSAIDs causes induction of mitochondrial injury in hepatocytes through uncoupling of oxidative phosphorylation and production of reactive metabolites that covalently bind to critical cellular proteins. In this study, we aimed to investigate the biochemical effects of antioxidants on indomethacin- induced liver injury in rats. Male Sprague-Dawley rats were randomly distributed into four groups. Group I; intact animals. Group II; control animals receiving vitamin C (vit C) (100 mg/kg), vitamin E (vit E) (100 mg/kg), beta-carotene (15 mg/kg) and sodium selenate (Se) (0.2 mg/kg) orally for 3 days orally. Group III; animals receiving 25 mg/kg indomethacin. Group IV; animals receiving vit C, vit E, beta-carotene and Se orally for 3 days (in the same dose and time), 2 hour prior to the administration of indomethacin. 6 hour after indomethacin administration all the animals were sacrificed. Liver tissue samples were taken and homogenized in 0.9% saline to make up to 10% homogenate. The homogenates were used for determining protein and protein carbonyl levels and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, xanthine oxidase, adenosine deaminase and myeloperoxidase activities. Liver protein carbonyl content and all of the enzyme activities were increased in indomethacin group. Treatment of indomethacin group with combined antioxidants reversed the increase of these biochemical parameters. In conclusion, the results of the present study indicate that treatment of combined antioxidants protect liver tissue against indomethacin-induced damage.

P08-34
All-trans-retinoic acid supplementation is effective to reduce hyperoxia-induced oxidative stress in mice brain

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Brain is very sensitive organ against oxidative stress due to containing a large amount of polyunsaturated fatty acids and exhibiting low antioxidant activity. This study investigated an effect of all-*trans*-retinoic acid (RA) on antioxidant activity of mice brain under hyperoxia-induced oxidative stress. Adult C57BL/6J mice were divided into 4 groups. Two groups were given daily intraperitoneal injections of peanut oil/dimethylesulfoxide (PoDMSO) mixture and 50 mg/kg RA dissolved in PoDMSO (RA-PoDMSO). The remaining two groups were treated in the same manner as already described following hyperoxia (100% oxygen) for 72-hrs: PoDMSO and RA-PoDMSO. The treatments were given as a daily 50 µl intraperitoneal injection for 12 days, with a 2-day break on days 6 and 7. Biochemically, lipid peroxidation (LPO) and glutathione (GSH) levels as well as activities of several antioxidant enzymes to confirm oxidative stress were determined in mice brain. Brain of hyperoxic mice were characterized by both the elevated LPO levels and activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase enzymes, and the decreased GSH levels. RA administration to hyperoxic mice resulted in improving of biochemical alterations in the direction of reduced oxidative stress in the adult brain. We suggested that exogenous RA, small lipophylic molecule, is effective to reduce oxidative stress in the brain receiving only small lipophylic molecules due to the blood-brain barrier. Also, RA has direct antioxidative properties, due to the inhibition of GSH depletion in brain of hyperoxic mice.

P08-35
Follicular fluid lipid composition of women undergoing controlled ovarian stimulation

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Follicular fluid makes up the physiological milieu of the oocyte during its maturation, and reflects the hormonal and metabolic processes, which take place in the growing oocyte microenvironment before ovulation. It is important to know the constituents of reproductive tissues to establish the molecular factors that regulate oocyte development and maturation. Very little is known about the lipid composition of follicular fluid. The aim of this work was to characterize the lipid profile, including the major phospholipid constituents, of follicular fluid at the preovulatory stage in women undergoing controlled ovarian stimulation. The study population consisted on oocyte donors and patients with fertility problems at the Valencian Institute of Infertility in Bilbao (www.ivi.es). The protocol of the study was approved by the University Ethics Committee (CEISH) and informed consent was obtained from the participants. Women were subjected to a standard stimulated ovarian protocol to induce ovulation. Follicular fluid was recovered at oocyte retrieval after hCG administration. Lipids from follicular fluid were extracted with chloroform/methanol, and the lipid residue dissolved in toluene containing cholesteryl formate as internal standard. Lipids were separated by six step-wise thin layer chromatography (TLC). After spot visualization by charring, lipids were quantified by image analysis. The most abundant lipids in follicular fluid were cholesteryl esters (48%) and phsphatidylcholine (about 28%). Sphingomyelin also showed a high proportion (9%), comparing with other phospholipids (2.6% phosphatidylethanolamine, and 1.0% phosphatidylinositol). Phosphatidylserine was under the limit of detection. This work was supported by FIS/FEDER (PI11/02559), the Basque Government (Department of Education, Universities and Research, ref. IT514-10), and UPV/EHU (CLUMBER UFI11/20). The work was performed according to the UPV/EHU and IVI-Bilbao agreement (Ref. 2012/01).

P08-36
Anti-inflammatory effects of MeOH extraction from the Amomum tsao-ko (AOM) involves HO-1 induction via ROS/MAPK/Nrf2 signaling in RAW 264.7 macrophages

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As an attempt to search for bioactive natural products exerting anti-inflammatory activity, we have evaluated the anti-inflammatory effects of MeOH extraction from the *Amomum tsao-ko* (AOM) (Zingiberaceae) and its underlying molecular mechanisms in LPS–induced macrophages. AOM reduced the production of nitric oxide (NO), prostaglandin E_2 (PGE_2), interleukin-1β (IL-1β) and interleukin-6 (IL-6) induced by LPS in both RAW 264.7 macrophages and peritoneal macrophages. Consistent with these data, AOM reduced the expressions of inducible nitric oxide synthase (iNOS) and cycloxygenase-2 (COX-2) at protein level and iNOS, COX-2, IL-1β and IL-6 at mRNA level in LPS-induced RAW 264.7 macrophages. AOM also time- and concentration-dependently induced HO-1 protein and mRNA expression that is a potent anti-inflammatory molecule, accompanied by a parallel increase of expressions and nuclear translocation of NF-E2-related factor 2 (Nrf2), master regulator of antioxidant responses. Moreover, small interfering (si)RNA-directed targeting of HO-1 reversed the suppressive effect of AOM on LPS-induced NO production and iNOS expression. We also found AOM induced phosphorylation of mitogen activated protein kinases (MAPKs), p38, extracelluar regulated kinase (ERK), c-Jun N-terminal kinase (JNK) as well as increased intracellular reactive oxygen species (ROS) production. Especially, pharmacological inhibitors of ERK and JNK blocked AOM-induced HO-1. Furthermore, pretreatment with anti-oxidant N-acetylcysteine (NAC) also reduced AOM-induced phosphorylation of ERK and JNK and expression of HO-1, suggesting ERK and JNK were downstream effectors of AOM-induced ROS. Taken together, AOM exerts anti-inflammatory effects through the ROS/MAPK/Nrf2 signaling to the upregulation of HO-1 in LPS-induced macrophages.

P08-37
Characterization of antioxidant enzyme activities in different tumour cell lines

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Characterization of tumour cells and the knowledge of their features is an essential step to develop therapeutic strategies against cancer. The different tumour cell lines contain different levels of expression of particular proteins, which contribute to their unique characteristics. In this work we have assessed the basal activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione *S*-transferase (GST), and catalase from human hepatomas (CHL, HepG2, SK-HEP-1, HuH7, PLC/PRF/5), rat hepatoma (McA-RH7777), human melanoma (A375), and human cervical carcinoma (HeLa). Cells were cultured in the adequate medium at 37°C in humidified atmosphere with 5% CO_2/95% air. When cultures reached a 75% confluence, cells were harvested and lysed in liquid N_2. Cells corresponding to 3–5 independent harvests were pooled and protein concentration was determined by Bradford. The enzyme activities were measured spectrophotometrically from at least three independent pools of cells. Results showed that the basal activities of the antioxidant enzymes varied depending on the cell line. Thus, the highest GPx (92 ± 12 nmolh/mg prot) and GST (1131 ± 42 nmolh/mgprot) activities were found in rat hepatoma McA-RH7777 cells, while in the other tumour cell lines GPx activities were considerably lower. Intermediate GST activities were found in A375, SK-HEP-1 and CHL, and in HeLa cells GST activity could not be detected. It is noteworthy the dramatically low GST (5.0 ± 1.2 nmolh/mg prot) and GPx (4.9 ± 0.4 nmolh/mg prot) activities found in HepG2; by contrast, catalase activity was 2 to 4-fold higher in HepG2 and McA-RH7777 than in the other tumour cell lines.

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P08-38
Gender-related reference intervals for urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine adjusted to creatinine (8-oxodG/creatinine) determined with liquid chromatography–tandem mass spectrometry

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Although there are many nucleobase modifications, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is one of the dominant forms of oxidative modifications in DNA or on the free nucleotide form of guanine in the nucleotide pool. Urinary 8-oxodG is the potentially best marker for monitoring oxidative stress. Defining reference interval for urinary 8-oxodG is a prerequisite for its clinical use as biomarker. Intention was to contribute to the validation of 8-oxodG in spot urine samples by determination of reference interval in the well characterized healthy population, using highly sensitive liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS, Thermo Scientific, Waltham, Massachusetts, USA). Reference population consisted of 229 healthy subjects (130 males and 99 females), mean age 45.3 ± 9.52 years. The urinary 8-oxodG was determined using UHPLC-MS/MS. Analytical performances of the UHPLC-MS/MS: CVs within-day and between-day variations were 5.6% and 2.6% respectively; LOD and LOQ were 1.65 nM and 3.30 nM; mean recovery and relative accuracy were 96% and 97% respectively. Urinary creatinine level was higher in males (15.70 ± 6.49 mM) than in females (11.60 ± 6.85 mM), but no gender differences in 8-oxodG was observed. Upon the adjustment of 8-oxodG concentration to the urinary creatinine (8-oxodG/creatinine), higher values were obtained in females (1.38 ± 0.65 nmol/mmol) than in males (1.05 ± 0.48 nmol/mmol). Due to the log-normal distribution of 8-oxodG/creatinine in spot urine sample, gender-related reference interval (as the 2.5–97.5 percentiles) was 0.45–2.22 nmol/mmol for males, and 0.54–3.11 nmol/mmol for females. In conclusion, this study has established gender-related reference intervals for 8-oxodG/creatinine in spot urine determined by highly sensitive and specific UHPLC-MS/MS method. Determination of the reference interval will contribute to the full validation of 8-oxodG as oxidative stress biomarker in spot urine sample.

P08-39
Hypoxia-inducible factor (HIF)-1alpha is not required for hypoxia-mediated protection of hematopoietic stem cells to oxidative stress-induced cell death

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In this study, we demonstrate that hypoxia protects HSCs from oxidative stress-induced cell death. This conclusion was drawn by investigating the effects of hypoxia when HSCs were challenged with ROS increase *in vitro* as well as *in vivo* upon transplantation to mice. Oxidative stress was induced by treatment with BSO (buthionine sulfoximine), which depletes the cells from glutathione. In normoxia, the apoptotic induction by BSO could be blocked either with the well-known antioxidant *N*-acetyl-L-lysine or by overexpression of Bcl-2, suggesting that the mechanism of cell death is linked to the levels of ROS in the cells and to intact mitochondrial function. Trying to elucidate the molecular mechanisms, hypoxic preconditiong could not delay HSCs from oxidative stress insult when placed in normoxia. Similar, cells first incubated in normoxia for 24 hours and then transferred to hypoxia were not rescued, indicating that this is a rapid adaptation to the oxygen levels provided by the environment. We also determined the involvement of HIF-1alpha by overexpression and knockdown experiments of LSK cells from conditional knock out mice lacking HIF-1alpha. Surprisingly, these studies demonstrated that HIF-1alpha is not involved in oxidative stress resistance in hypoxia. However, FoxO3a and its target genes SOD2 and catalase involved in the decomposition of ROS were upregulated by hypoxia as well as by BSO treatment. These results imply an involvement of FoxO3a independently of HIF-1alpha in hypoxia-mediated protection to oxidative stress in HSCs.

P08-40
Why fatty liver does not respond to isquemic preconditioning?

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The number of donor livers is insufficient to cover the needs for liver transplants, which drives the use of suboptimal livers, like those that have an important accumulation of fat (fatty liver/steatotic liver). The steatosis makes the liver more sensitive to the damage that results from the isquemia-reperfusion (I/R) process, but also makes it less responsive to preconditioning protocols that normally make the livers more resistant to I/R damage. Our study shows that the activation of the transcriptional coactivator PGC-1alpha, in response to I/R, is necessary to protect the liver from I/R damage and mediates the activation of protection mechanisms following the isquemic preconditioning protocol. It also suggest that the reduced activity of PGC-1alpha in the steatotic liver could be responsible for its increased sensitivity to I/R damage and poor response to preconditioning protocols. These observations allow us to propose PGC-1alpha as a relevant therapeutic target whose activation in the donor livers could facilitate the clinical use of steatotic livers for transplantation.

P08-41
Analysis of molecular mechanisms mediating the effect of tyrosol on *C. elegans* longevity

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Extra virgin olive oil (EVOO) consumption has been traditionally related to a higher longevity in the human population. EVOO effects on health are often attributed to its unique mixture of phenolic compounds with tyrosol and hydroxityrosol being the most biologically active. Although these compounds have been extensively studied in terms of their antioxidant potential and its role in different pathologies, their actual connection with longevity had not been explored in depth. Recently, we utilized the nematode *Caenorhabditis elegans* to investigate the possible effects of tyrosol in metazoan longevity and we observed a significant lifespan extension in *wild type* nematodes at one specific tyrosol concentration, which also induced a higher resistance to thermal and oxidative stress and delayed the pharynx pumping rate in this model organism. In this study, we first used Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOF-MS) in order to to confirm that tyrosol is efficiently incorporated by the nematodes. Also, we have used *C. elegans* mutant strains for several genes which are known to regulate longevity in order to pinpoint possible genetic requirements for the effects of tyrosol on longevity. Our data reveal that tyrosol is efficiently taken up by the nematodes, indicating that it does not undergo significant metabolic transformations by living bacteria. In addition, our lifespan experiments with mutant strains revealed that components of the heat shock response and the insulin pathway might be implicated in mediating tyrosol effects in both, lifespan and stress resistance, while caloric restriction and sirtuins do not seem to mediate its effects. Together, our results point to hormesis as a possible mechanism to explain the effects of tyrosol on longevity in *C. elegans*, although a more thorough analysis including a genomics/proteomics approach and the utilization of a larger set of mutants, would be mandatory in order to establish the precise molecular pathways connecting tyrosol and longevity.

P08-42
Stress resitance proteins response to Aesculum Hippocastanum extracts

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Horse chestnut extract and its active component, saponin escin have been shown to be effective augmenting therapy in endotoxemia by exerting antiinflammatory and anti-edematous effects. It has been confirmed, that escin especially, attenuates the attraction of neutrophils, damage to veins and release of growth factors. Between additional, but useful effects belong ability to increase venous tone and hyaluronidase inhibition. It was observed, that the beneficial effect latter could constitute a limiting side effect.

In this study we focused on stimulation or inhibition of antioxidant enzyme activities representing an adaptive stress response to horse chestnut and escine different dose exposures as well on viability of different human cancer cell lines.

We observed nonmonotonic dose-response curves by treatment of both extracts known as hormesis. Hormetic response typically involve antioxidant enzymes so that observed changes in superoxide dismutase, glutathione peroxidase and glutathione reductase activities could serve an explanation either of known antioxidant or toxic/side effects. Results of our study indicates that two of concentrations used for whole extract would be involved in activation of cellular stress response pathways and caused reduction in cells survival. First in CEM, HeLa and MCF and second in Jurkat T cells. Similarly, escin doses causing sudden oxidative stress, according to antioxidant enzymes activities, significantly decreased viability of Jurkat, CEM, HeLa or second MCF-7 cells. Clearly, we can say that both doses of substances that reduce survival of the cell lines caused the activity of glutathione reductase was significantly low, which suggests the possibility of enzyme inhibition, but also the possible down-regulation of expression. Study was supported by VEGA 1/1236/12 and 1/0751/12.

P08-43
Study beermaking process in which this is loaded of melatonin

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Beer is a beverage associated with the diet of numerous people, it is derived from the cereals (barley or wheat), containing a great array of bioactive phytochemicals and nutraceutical compounds. Specifically, high melatonin concentrations have been found in beer. The beneficial effects of melatonin, contained in beer, on human health are derived from the responsible consumption of this product, providing antioxidant, immunomodulatory, anticancer and metabolic properties. The brands of beer with alcohol content are those that presented greater concentrations of melatonin and *vice versa*. Immunoprecipitation, ELISA and HPLC were combined for melatonin determination. In the present work, we have synthesized beer in order to know, for the first time, the beer elaboration steps in which it is loaded of melatonin. We concluded that the alcoholic fermentation step by yeasts had a marked influence on the melatonin formation.

P08-44
Assessing toxicity of two types of magnetite nanoparticles in human hepatocarcinoma cells

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Magnetite nanoparticles are widely used for various biomedical applications such as magnetic resonance imaging, target delivery of drugs or genes, magnetic cell separation or hyperthermia. For all considered applications, the nanoparticles (NPs) must be biocompatible and non-toxic. Our aim was to investigate the possible toxic effects of magnetite nanoparticles (MN) and sucrose-coated magnetite nanoparticles (SMN) on HepG2 cells. HepG2 cells were exposed to NPs (synthesized by co-precipitation method) up to 72 hour at NPs concentrations ranging between 0 and 120 μg/ml, and two concentrations (6 and 60 μg/ml) were chosen for the determination of ROS, GSH, MDA, AOPP and protein carbonyl concentrations. The viability of MN-treated cells decreased in a time dependent manner, reaching 60% of control value after 72 hour at 60 μg/ml, whereas SMN did not affect this parameter. ROS generation varied in a time and dose dependent manner in the case of both concentrations, but it was more pronounced for higher dose when after an increase by 106% and 83% for MN respectively SMN compared to control was noticed. At 60 μg/ml GSH levels decreased in MN ad SMN-treated cells by 50% respectively 30%, while MDA ones increased by 186% respectively 290% compared to control, after 72 hour. At lower dose these effects were less pronounced. A dose-dependent raise of AOPPs was observed up to 3 days, by 56% and 248% for MN respectively by 34% and 114% for SMN at 6 μg/ml and 60 μg/ml NPs. Protein carbonyls significantly increased by 228% respectively 137% in MN and SMN treatment after 72 hours at higher dose. Our results suggest that both types of NPs generated dose-dependent toxic effects on HepG2 cells by inducing oxidative stress and its related consequences but SMN were better tolerated.

P08-45
Mitochondria-targeted antioxidant SkQR1 prevents acute kidney injury after ischemia/reperfusion, rhabdomyolyisis and gentamicin toxicity

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Acute kidney injury (AKI) appears to be the essential risk-factor for surgical and pharmacological interventions. Mitochondria were found to be a key player in this pathology, being both the source and the target for excessive production of reactive oxygen species (ROS). The aim of current work was to investigate the ability of mitochondria-targeted antioxidant SkQR1 (a conjugate of rhodamine with plastoquinone) to prevent the AKI of different genesis. We chosed three models of AKI: kidney ischemia/reperfusion (I/R), glycerol-induced rhabdomyolysis and gentamicin-induced nephrotoxicity. Injection of SkQR1 before and after the onset of the pathology in case of I/R and rhabdomyolysis and before every gentamicin injection was found to rescue rats from the renal failure, assessed by serum creatinine and urea, diminished histological lesion and significantly lowered mortality. The increase of MDA both in whole kidney and kidneys mitochondria was observed after I/R and rhabdomyolysis. I/R led to the burst of ROS-production and mitochondrial membrane potential dissipation. Incubation of renal tubular cells with myoglobin had the same consequences. SkQR1 treatment before the onset of I/R or rhabdomyolysis normalized the level of ROS and lipid peroxidation products in kidney and decreased the cytochrome c level in the blood after rhabdomyolysis. SkQR1 was found to induce some elements of nephroprotective pathways such as an increase in erythropoietin levels and phosphorylation of glycogen synthase kinase-3β in the kidney. SkQR1 also normalized erythropoietin level lowered after I/R and gentamicin treatment.

We conclude that SkOR1 exhibit apparent nephroprotective effects in different models of AKI due to its antioxidative properties and induction of preconditioning pathways. Supported by RFBR (11-04-00771 and 11-04-01307)

P08-46
Comparative *in vitro* effects of selected plant extracts on glutathione and cancer cell viability

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Many plants have beneficial multifunctional properties derived from their specific, biologically active components. Mostly they are secondary metabolites, such as terpenoids, phenols, glycosides and alkaloids. Due to possible synergistic reaction between the components is still unclear which components of essential oils and extracts may act as antioxidants, antimicrobials, immunomodulators or stimulators of the endogenous digestive enzymes. According to *in vivo* experiments, selected extracts act favorably to the animal health when supplemented in amounts 0.05% (0.1%) into the feed.

We assessed the proposed antioxidant effects of compounds on activity of liver mitochondria superoxide dismutase, level of reduced glutathione es well as estimated the effect on cultured cancer cell lines *in vitro*. Effective doses of essential oils from Origanum vulgare, Salviae officinalis, and extracts from Eleutherococcus senticosus, Stevia rebaudiana were prepared by binary dilution.

In vitro observation of the activity of superoxide dismutase in liver mitochondria would allow one to summarise that decreased enzyme activities under expected prophylactic dose unambiguously points to decreased number of superoxide radicals. In dependence on other doses of acting substance used, the activities seem not to be affected, either positively or negatively. However, cultivation under two mentioned doses with oregano and salvia caused decrease in the viability of HeLa, CEM, MCF-7, A-549 and MDA cells. In addition, salvia lowered viability of Caco 2 cells. Eventual chemopreventive or chemotherapeutic effect of dose, which corresponds to about 0.1% could be associated with decreased levels of GSH, we measured. It is curious that a similar effect of siberian ginseng extract was observed at higher concentration. Stevia influenced viability of MDA and Caco 2, what is interesting, but possible beneficial effect began to manifest in dose bringing limitations in experiments *in vivo*.
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P08-47
Biological effects of levan on CaCo2 cell line

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Previous studies revealed that levan produced by the Zymommonas mobilis, an extremophyle bacterium, has antitumoral effects on HeLa cells. As a result, our aim was to investigate levan effects on another tumoral cell type, i.e. CaCo2 cells. For this, cells were exposed up to 72 hour to levan solutions with concentration ranging between 0.1 and 6 mg/ml. The viability of cells varied in a time and concentration dependent manner and the concentration of 2 mg/ml was chosen for the determination of oxidative stress parameters. The reactive oxygen species generated after expose increased by 80%, 220% and 500% after 24, 48 respectively 72 hour. The GSH level showed a slight decrease after 48 and 72 hour by 20%, which is correlated with malondialdehyde generation. The SOD activity decreased by 40% after 24 hour but increased by 30% later on, whereas CAT activity decreased by 30% after 24 hour and increased significantly by 70% and 30% after 48 respectively 72 hour. The GST activity increased after 24 hour of exposure by 40% and decreased later on reaching the control values, which can be correlated with high malondialdehyde concentration. The decrease of protein sulfhydryls, by 80% after the first 24 hour suggests that these groups are important in the antioxidant defense of $CaCo_2$ cell at the beginning of exposure. It seems that after 48 hour, GSH become a more important player. Also, advanced oxidation protein products were unchanged after 24 hour and raised by 80% later on. Our results show that levan induces an oxidative stress on $CaCo_2$ cells, which could induce several types of cell death.

P08-48
Silica nanoparticles induce oxidative stress in human lung fibroblasts

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Silica nanoparticles have a wide variety of applications in medicine and industry. However, the potential biological effects of SiO_2 exposure and the toxic mechanisms remain unclear. SiO_2 nanoparticles are photosensitive and produce reactive oxygen species in the presence of light. In our study, the primary amorphous nanoparticle size distribution was a lognormal function, in the range 3–14 nm, most of the nanoparticles measuring 5–8 nm. The aim of this study was to investigate biochemical effects induced by exposure to silica nanoparticles in MRC-5. The MTT test was used to assess cell viability of MRC5 cells treated with 12.5 μg/ml SiO_2 for 24, 48 and 72 hours. The reactive oxygen species (ROS), lipid peroxidation (MDA), reduced glutathione (GSH), advanced oxidation protein products (AOPP), protein carbonyls and sulfhydryls levels were evaluated. The viability of MRC-5 cells significantly decreased in a dose and time dependent manner. The oxidative stress at cellular level was proved by increased MDA by 47%, 66% and 50% and reduced GSH levels by 36%, 50% and 78% after 24 hour, 48 hour respectively 72 hours. The concentration of intracellular ROS levels increased significantly by 85%, 62% and 43% after 24, 48 respectively 72 hours whereas protein carbonyl groups were increased after 48 and 72 hour of treatment with 11% respectively 50% compared to control. Protein sulfhydryls levels significantly decreased by 17% respectively, 33% compared to control after 48 and 72 hour. At the same time, advanced oxidation protein products concentration increased by 31%, respectively, 76% in the same intervals. According to our results, MRC5 cellls could not efficiently counteract the oxidative stress induced by silica nanoparticles exposure.

P08-49
Antioxidative kidney protection strategy in experimental pyelonephritis *in vitro*

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Different *in vivo* models for pyelonephritis are widely used for investigating the mechanisms of inflammation and renal insufficiency development. However, the role of different cells in inflammation, cell-to-cell communication and other molecular mechanisms are likely to be difficult to study in a whole organism model. Therefore, we proposed an *in vitro* pyelonephritis model which consists in co-cultivation of rat tubular epithelium cells and activated rat blood leukocytes.

We observed the rise of reactive oxygen species (ROS) level in renal tubular cells after co-culturing with leukocytes that led to increased cell death level. Activation of leukocytes by bacterial lysate induced release of TNFα, with higher intensity in co-culture. Interestingly, kidney cells can also produce TNFα in presence of *E. coli* lysate. Increased expression of TLR2 on tubular cells' surface in the pyelonephritis model suggests the direct involvement of renal cells in the inflammation. Direct cell-to-cell contacts between tubular cells and leukocytes appeared to be necessary for the inflammation development.

As *in vitro* model could provide fast and simple way of screening new drugs, we tested three nephroprotective compounds: widely used antioxidant Trolox, mitochondria-targeted antioxidant SkQR1 (10-(6'-plastoquinonyl)decylrhodamine) and GSK-3β inhibitor – lithium chloride. All of them decreased ROS production and kidney cells death, but did not affect the TNFα levels. Thus, *in vitro* model allowed us to highlight the key role of oxidative stress in pyelonephritic renal damage. Furthermore, mitochondria were found to not only generate ROS, but also to regulate cell death, and that suggests new mitochondria-targeted kidney protection strategy.

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P08-50
Effect of corticosteroids on metabolomic profile of exhaled breath condensate in patients suffering from difficult-to-control asthma: use of LC-MS/MS and NMR metabolomics

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Asthma bronchiale is a chronic respiratory disorder, which, because of advances in recent medicine, can be controlled in most

cases with a suitable medication. Serious form of asthma can be effectivelly treated with corticosteroids. However, 5–10% patients still suffer from difficult-to-control asthma (DCA). It is believed that high levels of oxidative stress are key factor in pathogenesis of asthma. Information about the specific biomarkers of oxidative stress can be obtained by analysis of exhaled breath condensate (EBC). To determine the influence of corticosteroid therapy on metabolomic profile of EBC in DCA patients, LC-MS/MS and NMR spectroscopy were utilized. Therefore, we investigated the differences in the metabolites of EBC from DCA patients treated with oral corticosteroids (OCS), DCA patients treated with inhaled corticosteroids (ICS) and a control group. Our preliminary results indicated increased lipoperoxidation and blood eosinophilia from ICS group in comparison to OCS treated patients. All other measured markers were comparable between ICS and OCS DCA.

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P08-51
Kinetic changes in liver Nrf2 activity triggered by sub-chronic administration of iron (Fe)

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Liver preconditioning against ischemia–reperfusion (IR) injury is a major area of experimental research. Significant cytoprotection against rat liver IR injury occurs after exposure to sub-chronic Fe administration (50 mg/kg, six doses every second day). Taking into account that transient oxidative stress induction underlies Fe-preconditioning, kinetic changes in liver redox-sensitive transcription factor Nrf2 nuclear translocation (western blot) and total reduced glutathione equivalents (GSH) content were evaluated following each Fe–dextran (50 mg/kg i.p.) administration (six doses) to male Sprague-Dawley rats. Significant GSH depletion after 2 Fe doses was followed by progressive recovery (3–6 doses), was paralleled by enhanced nuclear Nrf2 content, and augmented nuclear-Nrf2/cytosolic-Nrf2 ratios. These early effects triggered by Fe, were accompanied by progressive increments in the cytosolic Nrf2 inhibitor Keap-1 content, suggesting Fe-induced dissociation of the Nrf2/Keap-1 complex, followed by Nrf2 nuclear translocation. Taking into account that the expression of antioxidant and cytoprotective proteins is subjected to Nrf2 regulation, these results suggest that the early activation of redox-sensitive transcription factor Nrf2 signaling pathway, may mediate the cytoprotective response against IR liver injury afforded by sub-chronic Fe administration. Supported by FONDECYT 1110006.

P08-52
Peripheral blood B-cells bind epitopes on oxidized low-density lipoprotein (oxLDL)

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Antigens associated with oxidative stress are also displayed on apoptotic cells and some microbes, serving as means to evade immune recognition. Humans are born with premade antibodies – so called natural antibodies (NAbs) – capable of binding to these conserved epitopes, but little is still known about the B cells producing them and in what other ways adaptive immune cells interact with these epitopes.

Using malondialdehyde-modified bovine serum albumin (MDA-BSA) and MDA-LDL as model ligands for oxLDL, we compared peripheral blood lymphocytes from umbilical cord blood, healthy adults and chronic lymphocytic leukemia (CLL) patients. The CLL samples include subset 1 patients, whose monoclonal antibodies bind MDA epitopes.

Flow cytometry analyses show that only CD19+ lymphocytes (i.e. B cells), bind MDA epitopes. MDA+ cells are more often IgM+, CD5+ and CD27- compared to MDA- B cells in healthy adults. In newborns, the MDA+ cells are preferentially IgM+CD27+. CD38 and CD43 expression did not correlate with binding to MDA adducts, nor did the IgM expression on CLL monoclonal B cells.

Regardless of normalization method, gene expression arrays implied four differentially expressed genes when comparing MDA-binding versus non-binding B cells from the same donors, namely LOC613266, DUSP5 that inactivates ERK1, CERS6 which has been implicated in cellular stress and apoptosis and a transcript (acc. no. AF211977) whose sequence is identical to DNA-binding protein SATB homeobox 1.

We show that B cells but not T cells interact with epitopes classically associated with innate immune responses and oxidative stress, and that MDA+ B cells differ significantly from the MDA- B cells in terms of gene expression and phenotype. The binding of MDA does not depend on antibody specificity.

P08-53
Biochemical markers of oxidative stress and heat shock protein adaptative modifications in the liver of *Cyprinus carpio* injected with fluorescent silicone quantum dots

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The potential of quantum dots (QDs) for imaging applications raised concerns about their health consequences. Our goal was to investigate the effects induced by Si/SiO2 QDs in the liver of *Cyprinus carpio* by measuring the advanced oxidation protein products (AOPP), protein carbonyl groups (PRCG), protein thiols, lipid peroxidation (MDA) and GSH levels. We also evaluated Hsp60 and Hsp90 mRNA expressions by qPCR. The fish were divided in two groups: IP injected with 0.7% NaCl and IP injected with 2 mg QDs suspension/kg body weight. After 1, 2 and 3 weeks from injection five fish from each group were sacrificed.The AOPP, PRCG and MDA levels showed similar evolutions. The highest values were registered one week after injection, with increases of 46% ($p \leq 0.001$), 31% ($p \leq 0.05$) and respectively 97% ($p \leq 0.01$) above controls. These markers levels diminished in a time dependent manner and after three weeks they reached values close to controls. Protein thiol and GSH levels were lowest one week after treatment and represented 76% ($p \leq 0.05$) respectively 57% ($p \leq 0.001$) of control levels. Interestingly, they showed a clear tendency to recover and reached 87% and 95% of control levels after three weeks. After 1, 2 and 3 weeks the relative expression ratios (R) of Hsp60 were 0.45 ± 0.03, 0.65 ± 0.06 and 0.72 ± 0.23. The Hsp90 Rs were 0.23 ± 0.006, 0.42 ± 0.01 and respectively 1.03 ± 0.03. These data could indicate alterations in protein turnover in the first weeks after injection. Our results suggest that on short term, Si QDs induce oxidative

stress in fish hepatic tissue, and also trigger an adaptive modulation of Hsp60 and Hsp90 expressions. However, after three weeks all studied stress markers and heat shock protein levels recover and tend to reach values similar to controls.

P08-54
Role of adiponectin in astrocyte cells submitted to oxidative stress

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Oxidative stress refers to the situation where antioxidant defenses are overwhelmed with oxygen-reactive species resulting in cellular damages evidenced in several neurodegenerative disorders.Astrocytes are a sub-type most abundant of glial cells in the central nervous system (CNS) which emerge as key players in motor neuron degeneration.Adiponectin is a protein that modulates a number of metabolic processes. Its functions in metabolism regulation are well described, but its role in the CNS inflammation is not well known. Here,we investigate the importance of adiponectin in astrocyte cells submitted to oxidative stress. We subcloned the cDNA of adiponectin in eukaryotic expression vectors and stably transfected mouse CLTT astrocyte cells. Overexpressing adiponectin cells, as monitored by Q-RT-PCR, as well as non-transfected cells were treated with hydrogen peroxide (H_2O_2). Relative toxicity was determined using trypan blue and MTT.Protein oxidation and reactive oxygen species (ROS) levels were quantified by measuring carbonyl content and rates of DCF oxidation, respectively.CLTT cell expression of SOD, iNOS, COX2 and Catalase was determined by Q-RT-PCR.We demonstrated that adiponectin overexpression in CLTT cells protects from oxidative stress-induced cellular damages, improve cell viability, and reduces intracellular ROS formation, carbonylated protein accumulation. Pro-oxidative enzymes iNOS, COX2 were inhibited in H_2O_2 treated cells while anti-oxidative enzymes catalase and SOD level was increased. *In vivo*, elevated adiponectin mRNA levels in the hippocampus were demonstrated in CD1 mice 3 days following an acute ip injection of trimethyltin, which induces hippocampal neurodegeneration. Our results suggest that adiponectin secretion by CNS could represent a mechanism to protect nervous system in oxidative stress and inflammatory situation.

P08-55
Vanadium pentoxide – diferential survival of three different strains of *Saccharomyces cerevisiae* is ensured by glutathione reductase and mitochondrial glutathione level

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Vanadium is a heavy metal present in the Earth's crust whose utilization by the industry of steel, pesticides and paints has increased in the last years, contributing to increase of its environmental level, making it a pollutant. Several studies reveal that vanadium causes genotoxic and mutagenic effects, although may be beneficial for some organisms, exerting antitumor effects by activating the signaling pathways, which lead to apoptosis or induces P450 enzymes. Following from the above, the main objective of this work was to evaluate the response of three *Saccharomyces cerevisiae* strains to vanadium pentoxide present in the culture medium. *S. cerevisiae* UE-ME_3, a wine wild-type strain from Alentejo, Portugal; Red fruit, a commercial strain used in Alentejo for wine-make, and BY4741 EUROCAST strain, grown at mid-exponential phase were inoculated in 2% glucose YEPD medium and incubated during 72 hour at 28°C, in the absence or presence of 2 mM V_2O_5. Aliquots of the cultures were then used to dry weight determination, and to obtain mitochondria, for glutathione and glutathione reductase (GR) activity determination, and cytosol for malonaldehyde (MDA) quantification. The results showed that 2 mM V_2O_5 has caused cell growth inhibition in all strains, effect less pronounced in the UE-ME_3 strain in which were not detected significant differences in MDA content between control and treatments ($p < 0.05$). In addition, it was also observed a significant decrease in glutathione reductase activity and mitochondrial GSH/GSSG ratio, in al strains treated with vanadium pentoxide being this effect less pronounced in the wild-type strain. The smallest decrease of reducing power (47%) detected in *S. cerevisiae* UE-ME_3 may justify the better survival to V_2O_5 of wine wild-type strain, probably due better capacity to regenerate GSH via glutathione reductase, an antioxidant enzyme involved in redox cycle of glutathione.

P08-56
Lindane toxicity: can glutathione reductase and glucose-6P-dehydrogenase of *Saccharomyces cerevisiae* UE-ME3 provide sufficient protection against cytoplasmic damages?

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Lindane is an organochlorine persistent insecticide, lipophilic, chemically and biochemically stable, detected in the atmosphere, groundwater, sediments and soil. The literature describes this compound as toxic and able to affect animal reproduction and development. The aim of this study was to evaluate the effects of lindane in the wild-type *S. cerevisiae* UE-ME_3 of Alentejo, Portugal, a unicellular eukaryotic organism, described as resistant to the presence of pesticides or metals. Cells at mid-exponential phase were inoculated in YEPD medium with 2% (w/v) glucose and incubated during 72 hour in a water bath with orbital shaking, at 28°C, in the absence or in presence of 5 and 50 μM lindane. Samples from each treatment were used to obtain growth curves and to prepare post-12 000 ***g*** supernatant, used for determination of glutathione and MDA content by fluorimetry as well as GR and G6PD activities by spectrophotometry. The exposure to lindane caused a shift in growth profile after 24 hour of culture, occurring a decrease of cell growth in the final of exponential phase and at stationary phase, indicators of growth disturbance caused by this xenobiotic. Cells grown in presence of lindane showed an increase of G6PD and GR activities proportional to the organochlorine level in the media ($r = 0.972$ and $r = 0.988$, respectively). This response seems explain, in part, the increase in glutathione reducing power detected in post-12 000 ***g*** supernatant of 50 μM lindane, as well as the absence of significant changes in cytoplasmic MDA level. The increase in the G6PD and GR activities may also be correlated with the excretion of insecticide to the vacuole or with the transference of reducing equivalents which assist to anabolic pathways involved in the maintenance of cell proliferation. Despite the harmful effects caused by lindane, the reducing power transferred by G6PD and GR enzymes appear to be sufficient to minimize cell

damages in the cytoplasm of *S. cerevisiae* UE-ME_3. The cell growth disruption can eventualy results from events at peroxisome and/or mitochondria.

P08-57
Differential growth inhibition of Saccharomyces cerevisiae UE-ME3 and BY4741 by titanium dioxide nanoparticles in heat-shock conditions depends on glutathione reductase activity

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Nanoparticles of titanium dioxide, widely used as pigments or cosmetics, are usually found in molecular size < 100 nm. Sometimes, their poor thermal stability and large surface area can be correlated with their reactivity to cause changes in gene expression that may be used as biomarkers of exposure and cytotoxicity. Although the nanomaterials may be useful in medicine, their exposition to air or ultraviolet radiation for time periods as short as 30 min, makes them redox actives agents that induce lipid peroxidation and glutathione depletion, cytotoxic responses, that cause changes in permeability or conductivity of biological membranes. The main purpose of this work was to compare the response of *S. cerevisiae* UE-ME_3, a wine wild-type strain and BY4741, an invitrogen strain, to TiO_2-NP exposition in heat-shock conditions. Cells growing at mid exponential phase in liquid YEPD medium with 2% (w/v) glucose, at 28°C, were dark-exposed to heat-shock of 40°C and 0.1 or 1.0 μg/ml of TiO_2-NP, during 200 min, prepared by sonication, at same temperature conditions. Samples of each treatment were used to obtain the post-12 000 ***g*** supernatant for determination of dry weight, proteins, and glutathione contents as well as GR and GPx activities. The results show that heat-shock (28/40°C) caused a significant decrease in proteins and dry weight of BY4741 and UE-ME_3 strains, response that was accompanied by an increase in glutathione content and GSH/GSSG ratio, more pronounced in the BY4741 strain as well as a decrease in GR activity, case more pronounced in wild-type strain. Exposure to titanium dioxide nanoparticles (0.1 and 1 μg/ml) in heat-shock conditions induced a significant decrease in biomass, of UE-ME_3 strain followed by a significant decrease of GR and GPx activities. The best survival of BY4741 cells to TiO_2-NP seems related with an increase of GR activity, directly dependent of exposure level ($r = 0.9996$). The modeling of GR activity seems critical to regulate the growth inhibition of proliferating cells by heat-shock and titanium dioxide nanoparticles.

P08-58
Isoproturon increases *Saccharomyces cerevisiae* UE-ME3 proliferation, blocking NADPH and pyruvate generation via cytosolic dehydrogenases

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The isoproturon (IPU) is an herbicide used in Autumn-Winter crops that often persists in soils and aquifers at levels considered toxic by European legislation. In eukaryotic cells the IPU can generate ROS and consequently oxidative stress and it may be involved in triggering of serious illnesses like cancer. Accordingly, it is urgent to find microorganisms useful in bioremediation of this phenylurea. The aim of this study was to evaluate the effect of IPU on survival and antioxidant response of wild-type *Saccharomyces cerevisiae* UE-ME_3 from Alentejo wines, Portugal, that present great resistant to metals and adverse cellar conditions. *S. cerevisiae* at mid-exponential phase were inoculated in absence (YEP) or presence of 2% glucose (YEP-D) liquid media without or with 100 μM isoproturon (YEP-IPU; YEP-D-IPU) and incubated at 28°C during 72 hour. The cultures were used to draw growth curves (OD; cfu) and to estimate dry weight as well as to prepare post-12 000 ***g*** supernatant for spectrophotometric determination of protein contents, CAT A, G6PD, and NADP-ME activities. Yeast cells grown in presence of IPU, using glucose as main carbon source or in glucose starvation conditions exhibit a similar growth pattern (OD). However, the number of viable cells (cfu) is greater whenever yeast cells were grown in presence of IPU, fact that reflects higher cell proliferation conditioned by IPU. The CAT A, G6PD and NADP-ME activities has showed higher values in *S. cerevisiae* grown in YEP-D medium than yeast cells grown in YEP-IPU media. The same response pattern was observed when compared YEP-D-IPU with YEP-IPU. Probably, this response can be related with a catabolic repression of oxidative metabolism concomitantly with a eventual enhances of glycolytic flux and fermentation even in the presence of oxygen, caused by IPU inhibition of cytoplasmic conversion malate-pyruvate of *S. cerevisiae* UE-ME_3, which conferred a selective growth advantage useful in bioremediation.

P08-59
Effect of oral vanadium supplementation on oxidative stress factors in the lung tissue of diabetic rats

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Oxidative stress in diabetes leads to a reduction in antioxidant capacity, which in turn results in the increase of free radicals, shifting the balance in the wrong way, thus leading to cell integrity damage which is one of the the main causes of diabetic complications. Vanadium and vanadium compounds are responsible for insulin-like activity and can mimic the action of insulin through alternative signaling pathways. The protective effect of vanadyl sulfate on some tissues has been demonstrated previously by our colleagues. The aim of the present study was to investigate the effect of vanadyl sulfate supplementation on the antioxidant system in the lung tissue of diabetic rats. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg body weight) to male Swiss albino rats. The rats were randomly divided into four groups: Group I, control; Group II, vanadyl sulfate control; Group III, STZ-diabetic untreated; Group IV, STZ-diabetic treated with vanadyl sulfate. Vanadyl sulfate (100 mg/kg) was given daily by gavage for 60 days. At the last day of the experiment, rats which were fasted overnight were sacrificed, lung tissue was taken, homogenized in cold saline to make a 10% (w/v) homogenate. Antioxidant enzymes, catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione-*S*-transferase, as well as carbonic anhydrase, myeloperoxidase and lactate dehydrogenase activities were determined in the lung tissue. It was shown that vanadium supplementation decrease antioxidant enzyme activities which were

elevated in the lung tissue of the untreated diabetic group. We suggested that vanadium compounds could be used as preventive, due to its antioxidant effect in order to attenuate diabetic complications.

P08-60
The effects of bioactive thiols on the activity of CuZnSOD of the patients with chronic obstructive pulmonary disease *in vitro* and *in vivo*

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In this research the effects of three bioactive thiols, glutathione (GSH), its analogue UPF17 (o-methyl-L-tyrosinyl-L-glutamyl-L-cysteinyl-glycine) (1,2) and *N*-acetylcysteine (NAC), on the activity of CuZnSOD of erythrocytes of patients diagnosed chronic obstructive pulmonary disease (COPD) has been investigated.

CuZnSOD (EC 1.15.1.1.) is a cytosol-locating metalloenzyme that protects cell from oxidative stress by catalyzing the conversion of superoxide radicals into oxygen and hydrogen peroxide. The enzyme is a homodimer and contains free cysteine (Cys 111 and Cys 6), which can be used in a formation of heterodisulfide bonds with low molecular weight thiols leading to the change in the activity of CuZnSOD [3].

CuZnSOD activity in red cells was significantly increased in COPD patients compared with the enzyme activity of healthy controls. The effects of GSH and UPF17 were tested in red cells' lysates *in vitro* at the different preincubation time. GSH and UPF17 with concentration 10 μmol GSH or UPF17/g Hb caused the inhibition of CuZnSOD by 24% and 31%, respectively, after 30 min preincubation.

The difference between the activity of CuZnSOD of erythrocytes before and after the NAC treatment *in vivo* was measured. COPD patients were treated with 600 mg of NAC per day. After 4 weeks of treatment the activity of CuZnSOD was decreased by the average of 22%.

In conclusion, all three antioxidative thiols caused the inhibition of CuZnSOD in the erythrocytes of patients with COPD.

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P08-61
Metabolic changes induced by resveratrol in tumoral cells

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Resveratrol (RSV) is a stilbenoid known to activate sirtuins and to show prolongevity effects (Baur *et al.*, 2006). It is known that the treatment of tumor cell lines with RSV produces a dose-dependent S-phase delay starting as early as 6–12 hours of incubation. Cell cycle distribution seems to return to normal values after 48 hour of treatment depending on the dose of RSV. After this delay, metabolism of tumoral cells has passed from glycolitic to more respiratory metabolism. We have previously shown that RSV increases mitochondrial mass in several cell types including tumoral cells such as the human epithelial cervical cancer cell line HeLa (Baur *et al.*, 2006). RSV increased oxygen consumption in glycolitic HeLa cells accompanied by the modification of the activity and protein levels of electron transport chain components. Further, RSV induced a significant increase in β-oxidation. This effect was accompanied by increases in b-oxidation-related protein levels such as CPT-1 and CPT-2. Further, RSV also modified levels of pyridine nucleotides in cells by decreasing the ratio $NADH/NAD^+$. Although RSV is considered an antioxidant, treatment of HeLa cells with this compound increases ROS levels in a time and dose-dependent mechanism. This increase in ROS levels was produced in parallel of changed found in mitochondria. All this process was accompanied by the induction of autophagic mechanisms as determined by the increase in autophagy protein levels such as Beclin-1 or ATG-3. Our results indicate that RSV affects cell cycle progression at the same time that it is modifying the metabolism of tumoral cells from glycolysis to respiration. In this process, mitochondrial remodeling by induction of biogenesis at the same time than autophagy is involved. Baur *et al.* Nature 2007;444(7117):337–342.

P08-62
Molecular determinants of substrate specificity in a family of oxygen and/or nitric oxide reductases

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Herein we report studies on the molecular determinants of substrate selectivity in the oxygen-reducing flavodiiron protein from the anaerobic protozoan pathogen *Entamoeba* (*E.*) *histolytica.*

Flavodiiron proteins (FDPs) are a family of oxygen and/or nitric oxide reductases of prokaryotic origin. Despite extensive structural and functional data gathered on FDPs from several sources, the molecular details which underlie their substrate selectivity remain elusive. We have compared sequence and structural data on two FDPs with opposing substrate preferences, namely the O_2-reducing *E. histolytica* EhFdp1 and the FDP-domain of the NO-reducing *Escherichia coli* flavorubredoxin (EcFdp-D), and observed subtle differences in two positions in the environment of the non-heme diiron centre at the active site.

We generated single and double mutants of EhFdp1 replacing the residues in these positions with their equivalent in the NO-reducing EcFdp-D and compared their kinetic and thermodynamic properties by stopped-flow absorption spectroscopy, O_2/NO amperometry, and potentiometric-coupled spectroscopic studies.

As compared to WT EhFdp1, the mutants exhibit differences in their reactivity towards oxygen and interestingly appear more reactive towards NO, in line with the working hypothesis.

Altogether, the data provide further clues into the molecular details that determine the substrate preference in this family of detoxifying enzymes, which afford protection of pathogenic microbes against oxidative and/or nitrosative stress.

P08-63
Analysis of the composition of NFκB complexes mobilized by the antitumor drug doxorubicin

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Doxorubicin (DOX) is a potent antineoplastic drug used in the treatment of a wide variety of malignancies. However, its clinical use is limited by a significant dose-dependent cardiotoxicity, nephrotoxicity and hepatotoxicity. DOX-induced toxicity is generally mediated through the generation of reactive oxygen species, which alter intracellular signalling. In previous studies we have shown that DOX induces the translocation of NFκB into the nucleus and its DNA binding to consensus sequences in primary cultures of rat hepatocytes. However, DOX treatment resulted in the repression of genes under NFκB control. There are several forms of NFκB, including homodimers and heterodimers of p65, p50/p105, c-rel, relB, and p52/p100. Homodimers of p50 and p52 are considered to be inhibitory because they contain DNA-binding motifs without a transcription-activating domain. The present study was undertaken to define the composition of NFκB complexes mobilized by DOX. For this purpose electrophoretic mobility shift assays were performed with nuclear extracts from cultured hepatocytes treated without and with DOX for 6 hour. For identification of NFκB subunits, samples were incubated with antibodies against p65, p50, p52, c-rel, relB, and bcl3. Shift and supershift generated bands suggest that DOX-mobilized NFκB complexes are constituted by p50 homodimers, p50/p65 heterodimers, and c-rel containing dimers.

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P08-64
Mitochondrial dysfunction and ROS induce inflammation in Fibromyalgia patients

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Objective: Mitochondrial dysfunction has been implicated in the pathophysiology of Fibromyalgia (FM). Inflammation has been also hypothesized in FM. We will study the possible relationship between mitochondrial dysfunction, oxidative stress and inflammation in FM.

Methods: Mitochondrial dysfunction was studied assaying Coenzyme Q_{10} (CoQ_{10}), mitochondrial ROS production and mtDNA contents in blood mononuclear cells (BMCs). Serum TNF-alphaand gene expressionwas assayed as inflammatory mediator in patients. Clinical symptoms were evaluated using Visual Analogical Scale of pain (VAS), and Fibromyalgia Impact Questionnaire (FIQ). CoQ_{10} deficiency was induced in healthy cells and mice to evaluate TNF-alpha release.

Results: BMCs from FM patients showed reduced level of CoQ_{10}, mtDNA, and high level of mitochondrial ROS and TNF-alpha serum and transcript levels. A significant negative correlation between CoQ_{10} and TNF-alpha levels ($r = -0.588$; $p < 0.01$), and a positive correlation between ROS and TNF-alpha levels ($r = 0.791$; $p < 0.001$) were observed accompanied by significant correlation of VAS with TNF-alpha serum and transcript levels ($r = 0.4507$; $p < 0.05$ and $r = 0.7089$; $p < 0.001$, respectively). TNF-alpha release was observed in an *in vitro* and *in vivo* CoQ_{10} deficiency model.

Conclusions: Our data evidence that mitochondrial dysfunction has an important role in FM. Inflammation is a mitochondrial dysfunction-depended event implicated in the pathophysiology of FM.

P08-65
Functional validation of LexDBDGim chimeras in *Saccharomyces cerevisiae*

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GimC/Prefoldin is a hetero-oligomeric complex involved in cytoskeleton biogenesis. It is composed by six different subunits belonging to α (Gim2/Pfd3 and Gim5/Pfd5) and β (Gim1/Pfd6, Gim3/Pfd4, Gim4/Pfd2 and Gim6/Pfd1) classes. This complex interacts with actin and tubulin nascent polypeptides while associated with ribossome and deliver them to chaperonin CCT/TriC. In *Saccharomyces cerevisiae*, the absence of each subunit leads to different phenotypes, for instance, in the presence of osmotic and oxidative stress agents.

In order to identify by two-hybrid system targets that directly interact with Gims and support the stress phenotypes, this work aimed the functional validation of all Gims in *Saccharomyces cerevisiae*. One-hybrid assays with each Lex_{DBD}Gim (Lexgim) chimeras and *LacZ* reporters containing different promoters were performed. The obtained results allow the validation of the Lexgim chimeras in the context of two-hybrid assays. However, since the Gim/target interactions may occur only in stress conditions, the one-hybrid assays are currently being performed upon stress stimuli.

The results also led to the identification of specific promoters in which, under optimal growth conditions, Lexgim chimeras do not operate as activators neither repressors of transcription mediated by RNA polymerase II. In previous work developed in our laboratory with gim-null mutants, no correlation was found between the stress phenotypes and cytoskeleton defects and it was hypothesized that the phenotypic differences could be due to differential expression of specific stress genes. Since literature data does not exclude the presence of Gim subunits in the nucleus, the potential of Lexgims to regulate transcription mediated by RNA polymerase II will be re-evaluated under stress stimuli.

P08-66
Liver proteome expression profile in diabetic obese subjects

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Type 2 diabetes mellitus (T2DM) increases the risk of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in obese patients. To better understand the association between T2DM and NAFLD, global changes in protein expression in diabetic and non-diabetic obese subjects were assessed by a proteomic approach. Tenliver samples were obtained from diabetic and non-diabetic morbid obese subjects. Histological analysis was used to evaluate hepatic steatosis and the degree of anatomopathological alteration. Anthropometric and serum parameters were also determined. Changes in protein expression were analyzed by 2D electrophoresis combined with MALDI TOF mass spectrometry. Levels of glutathione, carbonyl and 4-HNE protein adducts were used to assess oxidative stress status. Of 850 proteins analyzed, 33 were differentially expressed in T2DM obese subjects. Of these, 27 were unequivocally identified by mass spectrometry. Analysis of protein sets revealed patterns of decreased abundance in mitochondrial enzymes (short-branched chain specific acyl-CoA dehydrogenase, hydroxyacyl-CoA dehydrogenase, hydroxymethylglutaryl-CoA synthase) proteins involved in methionine metabolism (*S*-adenosylmethionine synthase, glycine n-methyltransferase adenosylhomocysteinase) and oxidative stress response (prohibitin, peroxiredoxin, glutathione *S*-transferase). Accordingly, T2DM subjects showed decreased levels of glutathione, the antioxidant byproduct of methionine metabolism via the transsulfuration pathway, and higher levels of protein and lipid oxidative damage. Changes in detoxifying enzymes (carboxylesterase-1, glyoxalate reductase), carbohydrate metabolism (fructose-biphosphate aldolase B), proteasome subunits and retinoic acid synthesis (retinal dehydrogenase) were also found.The results suggest alterations in mitochondrial function and methionine metabolism as potential contributing factors to increased oxidative stress in liver of obese diabetic patientswhich may be influencing the development of NAFLD and NASH.

P08-67
Gender differences in the fetal to neonatal transition: clinical and experimental data

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Mortality and morbidity in the neonatal period is significantly lower in female than in male gender. However, very few studies in the literature have approached the importance of gender in the process of postnatal adaptation. In immature sheep it was shown that blood pressure was increased in response to antenatal steroid exposure and that sex-specific effects on renal function also existed. In humans, we showed that female preterm had less oxidative stress and increased antioxidant enzyme activity (AEA) and better clinical outcomes than did male infants. These results were in accordance with a previous report about a significant gender-specific difference favouring girls of the beneficial effects derived from avoiding hyperoxia. We also found that antenatal corticoid reduced postnatal oxidative stress and increased AEA more effectively in female than in male preterm. In addition, sex-dependent pulmonary vascular reactivity following short-term exposure to hyperoxia in newborn rats was evaluated. During the first week of life response to thromboxane was increased in vessels of O_2-exposed male rat pups whereas the opposite effect was observed in females. The enhanced agonist-induced force of hyperoxia-exposed male pulmonary arteries was suppressed by either superoxide or peroxynitrite scavengers. These changes were associated with a significant increase in lung superoxide dismutase (SOD) activity and H_2O_2 content in female but not in male rats. We conclude that fetal-to-neonatal transition is better faced by the female gender. Our findings contribute to explain the pathophysiologic events leading to an increased pulmonary hypertension and mortality in male neonates.

P08-68
The effect of melatonin on the liver of rats exposed to microwave radiation

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Oxidative stress is the key mechanism of the microwave induced tissue injury. Melatonin, a lipophilic indoleamine primarily synthesized and released from the pineal gland is a powerful antioxidant and imunomodulator. We aimed to clarify if melatonin treatment may favorably impact on liver tissue injury in rats exposed to microwave radiation. We explored the effects of a 6-week daily (2 mg/kg i.p.) administration of melatonin in adult rats exposed to microwaves (4 hours/day) produced by a mobile test phone. Wistar rats were divided in four groups: I (control) – rats treated with saline, II (Mel) – rats treated with melatonin, III (MWs) – microwave exposed rats, IV (MWs + Mel) – microwave exposed rats treated with melatonin. We evaluated oxidative stress parameters (malondialdehyde and carbonyl group content), antioxidant enzyme activity (catalase) and activity of enzyme xanthine oxidase, which has pro-oxidative effects. Exposure to microwaves caused an increase in malonyldialdehyde ($p < 0.01$) and protein carbonyl content ($p < 0.001$). Furthermore, decreased catalase ($p < 0.05$) and increased xantine oxidase activity ($p < 0.05$) were also observed. Melatonin treatnemt led to malondialdehyde decrease ($p < 0.05$), but surprisingly had no effect on other analyzed parameters. In conclusion, melatonin exerts certain antioxidant effects in the liver of rats exposed to microwaves, by diminishing intensity of lipid peroxidation.

P08-69
Oxidation of p53 family proteins modulates DNA binding properties *in vitro* and *in vivo*

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The p53 gene family (p53, p63 and p73) has important functions in many cellular processes such as DNA repair, cell cycle control or apoptosis. All p53 family members are important for the pre-

vention of cancer, but only p63 and p73 are crucial for normal development. While p63 and p73 proteins have been shown to transactivate a number of p53 target genes in common, both p73 and p63 also exhibit promoter selectivity and transactivate a number of unique target genes. Proteins encoded by these three genes share the same modular organization which consists of an amino-terminal transactivation domain, a central sequence-specific DNA binding domain and a C-terminal tetramerization domain. As expected, the highest homology between the p53 family members is found in the DNA-binding domain. DNA binding of p53 is known to be strongly inhibited by oxidative stress. The p53 family core domains hold a zinc atom that is critical for their DNA binding. It has been shown that inhibition of p53-DNA binding by diamine oxidation is reversible in the presence of Zn^{2+} *in vitro* (1). In this study we used diamide and peroxide agents for oxidation of cysteine residues in the p53 family core domains (2). The p53 family proteins were bound to linear DNA fragments or oligonucleotides containing the p53 response elements before or after treatment with agents. Our data show that the binding properties of all three proteins are inhibited by these agents and the Zn^{2+} ions influence reversibility of p53 family protein oxidation.

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P08-70
Effects of soy isoflavone Genistein on cell cycle and oxidative stress in breast cancer cell lines with different ERα/ERβ ratio

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The beneficial effects of soy consumption on breast cancer are controversial. Genistein (GEN) is a soy isoflavone with estrogenic activity, since it exerts its function on cells through estrogen receptors (ER). There are two ER subtypes, ERα and ERβ, associated with proliferative and with cytostatic and cell differentiation processes, respectively. 17β-estradiol (E2) induce oxidative stress through decreasing antioxidant enzymes activity in breast cancer cell lines with a high ERα/ERβ ratio, but not in low ERα/ERβ ratio cell lines. GEN has greater affinity to ERβ, halting cell cycle and inducing apoptosis. The aim of this study was to determine GEN effects on cell cycle and oxidative stress in breast cancer cell lines with different ERα/ERβ ratio, compared with E2. Sirtuin 3 (Sirt3) levels were also studied due to their role in oxidative stress because it modulates antioxidant enzyme activities such as superoxide dismutase. Two breast cancer cell lines with different ERα/ERβ ratio were studied: MCF7 (high ERα/ERβ ratio) and T47D (low ERα/ERβ ratio). Treatments were vehicle (0.001% DMSO), E2 (1 nM) and GEN (1 μM) during 48 hours. Cell cycle, ROS production, antioxidant enzyme activities and Sirt3 protein levels were analysed. In both cell lines GEN treatment produced a greater number of cells in G_0/G_1cell cycle phase, lower ROS production and greater increase in antioxidant enzymes activity and Sirt3 levels than E2 treatment. These effects were more pronounced in T47D than in MCF7. In conclusion, our study demonstrates that GEN produces a decrease in oxidative stress due in part to the increase of antioxidant enzyme activities and Sirt3 expression that could be responsible to their cytostatic effects. These effects were stronger in cell lines with higher levels of ERβ.

P08-71
Vanadium as preventive on oxidative stress factors in stomach tissue of STZ-diabetic rats

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The protection of cell integrity is important specially in diabetes where oxidative stress leads to complications in all tissues. It is reported that vanadium compounds are glucose-lowering agents which are shown to mimic insulin both *in vitro* and *in vivo*. Despite numerous studies, the mechanism(s) by which vanadium mediates its metabolic effects *in vivo* are still not completely understood. In this study, we aimed to investigate the effects of oral vanadium sulfate supplementation via antioxidative system in experimental diabetes, in the stomach tissue of streptozotocin (STZ)-diabetic rat models. Male Swiss albino rats were used throughout the study, and they were rendered diabetic by intraperitoneal injection of STZ at 65 mg/kg body weight. Vanadyl sulfate was given every day by gavage at a dose of 100 mg/kg. The experimental groups were as follows: N: normal control group; N + Va: normal animals given vanadyl sulfate; D: diabetic animals; D + Va: diabetic animals given vanadyl sulfate. The experimental period lasted 60 days at the end of which rats were killed under anesthesia by cardiac puncture and stomach tissues were taken. 10% (w/v) homogenates were prepared by homogenizing tissues in cold 0.9% NaCl, by means of a glass homogenizer. In order to evaluate the effect of vanadium on the antioxidant system, catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase, glutathione-*S*-transferase, myeloperoxidase activities were measured in stomach tissue homogenates. Carbonic anhydrase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities were also evaluated for the role of vanadium on carbohydrate metabolism. The activities of all enzymes were increased in the diabetic group and it was shown that vanadyl sulfate administration decreased significantly enzyme activities. We can conclude that vanadium exerts a preventive effect on the damage provoked by diabetes on tissues, via its antioxidant properties.

P08r-72
Induction of endogenous antioxidant enzymes by endurance exercise and resveratrol treatment in the gastrocnemius muscle of old mice

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Human sedentary lifestyle is the main responsible of the increasing risk for cardiovascular diseases, diabetes, obesity and other metabolic diseases. Then, it is very important to find some alternative therapies based on exercise, balanced diets and supplementation with bioactive compounds including those with antioxidant properties to ameliorate the life expectancy. We propose that moderate exercise and resveratrol can modify the expression pattern of genes involved in antioxidant protection in the muscle during aging, thereby improving cell activity and pre-

venting the accumulation of oxidative damage. All experiments were conducted on old mice (24 month) C56BL/6 male mice. Animals were divided in four groups: ethanol control, ethanol trained, resveratrol and resveratrol trained. During six months all controls received water with vehicle (ethanol), and all R animals received water with 10 mg/100ml of *trans*-resveratrol (10–20 mg/kg body weight). Following four months, trained mice were exercised at 15 m/min for 20 min per day, 5 day per week for a total of 6 weeks. To determine the expression profiles of genes related to oxidative stress, RT2 Profiler PCR Array for Real-time PCR was performed. Endurance exercise and resveratrol treatment increased physical performance in old mice. Both trained and resveratrol groups also showed an increased expression of the antioxidant enzymes, such as Gpx, Txnrd, Prdx, SOD3, Nox4 involved in biological redox processes and oxidative stress response, as well as Xirp1 involved in protecting actin filaments from depolymerization. The current study shows that endurance exercise and resveratrol treatment increase physical performance in old mice and mediates improvement the antioxidant enzymes protecting skeletal muscle against oxidative stress.

P08-73
Human erythrocyte's *O*-beta-*N*-Acetyl-glucosaminidase as biomarker of oxidative stress in erectile disfunction patients

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Background: Erectile dysfunction (ED) is the inability to achieve or maintain erection sufficient for satisfactory sexual intercourse. Although ED is a multifactorial process, vascular disease of the penile arteries is an important cause in up to 80% of cases. Recent studies underline that the Oxidative Stress (OS) plays an important role in pathophysiological process of ED as well the production of NO by endothelium Nitric Oxide Synthetase (eNOS). Interestingly eNOS is activated by phosphorylation at Ser-1177 and inactivated by O-GlcNAcylation at the same site by O-GlcNActransferase (OGT). A third enzyme is involved in this mechanism, the O-β-*N*-Acetylglucosaminidase (OGA), that split out O-GlcNAc residues allowing Ser-1177 ready for phosphorylation. Cellular levels of O-GlcNAcylated proteins are related to OS, consequently ED is a disease where dysfunctional eNOS O-GlcNAcylation seems to have a crucial role. The presence of NOS (related to vascular one) and OGA was recently showed in human erythrocytes (RBC). These observations allow us to assume that what happens in RBC might reflect what occurs in the endothelial cells (that is difficult to study) and to undertake a research to elucidate the role of RBC-OGA as a possible specific markers of OS in ED patients.
Methods: In 39 ED patients (50.1 ± 16 years) and 30 matched controls (52.9 ± 10 years) we studied plasma antioxidant total defenses by Lag-time method, cytosolic OGA, cytosolic and membrane Hexosaminidase (Hex), membrane β-D-glucuronidase (GCR) and α-D-glucosidase (αGLC) activities by fluorimetric assay.
Results: Compared to controls, patients Lag-time values are significantly decreased ($p < 0.01$) as well both cytosolic OGA and Hex activities ($p < 0.001$). Instead plasma membrane activities of Hex, GCR and αGLC are significantly higher ($p < 0.01$).
Conclusions: These preliminary data confirm the strong OS in ED patients, the specific role of OGA as early OS biomarkers and suggest its use in monitoring ED patients who undertaken a nutritional/pharmacological antioxidant therapy in the attempt to correct oxidative stress.

P08-74
High spin nitrated cytochrome *c* is specifically degraded inside the cells

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Cytochrome *c* (C*c*), a small protein localized in the intermembrane mitochondrial space (IMS), has a dual role in cell life (oxidative phosphorylation, OxPhos) and death (apoptosome assembly). Reactive Nitro/Oxygen Species (RNOS) are collaterally produced during OxPhos but are neutralized by the cell detoxifying system. Under metabolic unbalance, however, the RNOS level rises up and induces nitration of proteins on tyrosine residues. Among mitochondrial proteins, C*c* is one of the main targets to be nitrated. Actually, three out of its five tyrosine residues (at positions 67, 74 and 97) are nitrated in different tissues, but there is no any biological evidence of nitration of Tyr46 or Tyr48 *in vivo*, although they are both well-exposed to solvent.

Nitration of Tyr46 or Tyr48 in monotyrosine mutants of C*c* – with all tyrosines but one replaced by phenylalanines – turns the heme iron into a high spin state, with negligible effects on the rate of electron transport to C*c* oxidase 1) but yielding a non-functional apoptosome (1), unable to activate caspases. In addition, nitration of Tyr46 or Tyr48 increases the peroxidase activity (1) of high-spin C*c*. In cardiolipin-harboring liposomes, the affinity of such nitrated C*c* species to membranes is impaired, so as to fill the IMS pool of C*c*. This finding correlates with the fact that only the high-spin iron nitrated forms of such C*c* mutants are specifically degraded in *Jurkat* cell extracts devoid of proteases inhibitors (2), suggesting that nitration could act as a signaling mechanism.

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P08-75
Influence of thyroid hormone (T3) administration on rat liver Nrf2 and AMPK signaling pathways

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The calorigenic action of T_3 is associated with the generation of reactive oxygen species (ROS) and hepatoprotection against ischemia-reperfusion liver injury. The administration of T_3 to Sprague-Dawley rats (0.1 mg/kg ip) enhanced the cytosol-to-nuclear translocation of liver Nrf2 (Western blot), a transcriptional factor activated by ROS. The levels of liver antioxidant proteins (glutamate-cysteine ligase, heme-oxygenase-1, and thioredoxin), phase-2 biotransformation enzymes (glutathione-*S*-transferase, epoxide hydrolase, and NADPH-quinone oxidoreductase), and phase-3 transporters (canalicular MRP-2 and sinusoidal MRP-4) controlled by Nrf2, were significantly ($p < 0.05$) increased. In addition, preliminary studies revealed activation of

liver AMP-activated protein kinase (AMPK) by T_3, as evidenced by the significant enhancement ($p < 0.05$) in the phosphorylated-AMPK/non phosphorylated AMPK ratios, enzyme augmenting cellular ATP turnover. It is concluded that the administration of T_3 confers a greater antioxidant and detoxifying potential to the liver associated with the preconditioning action of the hormone. This effect of T_3 is energetically supported by AMPK activation to repair or synthesize biomolecules and trigger cell proliferation, which are affected by ischemia-reperfusion (supported by FOND-ECYT 1120034).

P08-76
Effects of vitamin U (*S*-methyl methionine sulphonium chloride) on valproic acid induced liver injury in rats

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Valproic acid (2-propyl-pentanoic acid, VPA) is recommended for several therapeutic purposes. For many years it has been used in treatment of epilepsy and considered to be a safe antiepileptic drug in a wide range of epileptic disease. VPA is usually well tolerated, but serious complications including hepatotoxicity, hyperammonemic encephalopathy and teratogenicity may occur. Vitamin U (Vit U), methylmethionine sulphonium chloride, is a vitamin-like active substance. In this study, we aimed to investigate the effects of Vit U on VPA-induced liver damage. Female Sprague Dawley rats were randomly divided into four groups. Group I; intact (control) animals. Group II; control animals given Vit U (50 mg/kg/day) for fifteen days. Group III; animals given only VPA (500 mg/kg/day) for fifteen days. Group IV; animals given VPA + Vit U (in same dose and time). Vit U was given to rats by gavage and VPA was given intraperitoneally. On the 16th day of experiment, all the animals were fasted overnight and then sacrificed under ether anesthesia. Liver tissues were taken and homogenized in 0.9% saline to make up to 10% homogenate. Liver aspartate and alanine transaminase, alkaline phosphatase, lactate dehydrogenase, myeloperoxidase, sorbitol dehydrogenase, glutamate dehydrogenase and xanthine oxidase activities and lipid peroxidation levels were increased and paraoxonase activity and glutathione levels were decreased in VPA group. Treatment with Vit U reversed these effects. These results demonstrated that administration of Vit U is a potentially beneficial agent to reduce the liver damage in VPA induced hepatotoxicity, probably by decreasing oxidative stress.

P08-77
Antioxidant contents, antioxidant activities and UV-absorbing properties of fifteen plant species native to Thailand

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Antioxidant is critical defense mechanism by protecting damage effects of free radical. Ultraviolet (UV) radiation is one source of free radical generation, thus causing harm to the living systems. The aim of the present study was to investigate the antioxidant contents, antioxidant activities and UV-absorbing properties of fifteen species of Thai plants. All Thai plants were exhaustively extracted by petroleum ether, dichloromethane and ethanol respectively. Antioxidant contents were determined by Folin-Ciocalteu and Aluminum chloride colorimetric method. Antioxidant activities were assessed by DPPH and ABTS assay. UV absorption properties were measured by spectrophotometer. The results showed that turmeric from dichloromethane fraction extract was shown to contain the highest amounts of total phenolic and total flavonoid contents (85.66 ± 2.75 GAE equivalents/g fresh weigh and 1092.71 ± 117.49 mg QE/g fresh weigh, respectively). Turmeric from dichloromethane fraction extract was also found to possess the highest antioxidant activity (85.30 ± 1.02% SC, IC_{50} = 141.78 μg/ml by DPPH assay and 93.60 ± 0.25% SC, IC_{50} = 88.05 μg/ml by ABTS assay). In addition, the maximum UVA (315–400 nm) and UVB (280–315 nm) absorptions were found in turmeric from ethanolic fraction extract and ginger from dichloromethane fraction extract, respectively. Taken together, these data highlighted the intriguing properties of turmeric extracts. Therefore, further studies on the molecular mechanisms of these extracts on preventing UV-induced cellular damage should be carried out in the near future.

P08-78
Heavy metal-induced alternative splicing of glutathione synthetase mRNA in the brain of common carp (*cyprinus carpio*)

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Introduction: Glutathione (GSH), synthesized by glutathione-synthetase (GSS), is a thiol-containing tripeptide that participates in the cellular defense against oxidative damage by free radicals. Oxidative stress plays important roles in pathogenesis, and a deficient GSS activity is therefore one of the major triggers in the onset of many diseases.
Results: cDNA clones carrying the coding region of *gss* and its splice variant *gss-a*, were isolated from a brain cDNA library. The *gss-a* transcript is characterized by the lack of a 259 nt segment corresponding to exons 4 and 5. The alternatively spliced product was found only in the brain of the unstressed animals, while *gss* mRNA was detected in all the tissues examined, with the highest level in the kidney. Cd^{2+} exposure induced *gss-a* formation, though the amount of *gss*-specific mRNA was not significantly influenced.GSS functions as homodimer in an ATP-dependent manner. The predicted GSS-A protein is most probably inactive catalytically since it lacks the complete ATP-binding domain, but it is still likely to be able to participate in dimer formation.
Materials and methods: The expression of gss/gss-a mRNA was assessed by reverse transcription coupled polymerase chain reactions (RT-PCR) in different tissues of untreated and Cd^{2+}-exposed common carp.
Conclusion: The *gss* splice variant of common carp is present in the brain under physiological conditions, and its level is significantly increased during Cd^{2+}-induced oxidative stress. An alternative splice variant of human *gss* mRNA has already been identified[1] and it has been demonstrated that the level of alternative splicing is increased in certain human carcinoma cell lines. Carcinogenesis is believed to be associated with enhanced formation of free radicals. The two known examples of elevated alternative splicing of *gss*-mRNA in evolutionarily distant species

suggest a role of GSS-A protein in the cellular defense against oxidative stress. Financial support from the TÁMOP-4.2.2/B-10/1-2010-0012 fund is gratefully appreciated.

Reference

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P08-79
Identification of pathogenic cholesterol oxidation products in hepatic tissue and bile of cholangiocarcinoma patients

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Oxysterols are oxidation products of cholesterol, which are generated by either cytochrome P450 (CYP) enzymes or reactive oxygen and nitrogen species. They are intermediates in bile acid synthesis as well asimportant signaling agents. However, some certain species are cytotoxic and carcinogenic. Oxysterols have been identified in bile in the setting of chronic inflammation. Interestingly, some species; 3-keto-cholest-4-ene (3K4) and cholestane-3β-5α-6β-triol (triol), which are carcinogenic, were indentified in hamster liver tissue of liver fluke-induced cholangiocarcinoma (CCA). Our previous study reported that liver fluke-induced oxysterols can generate DNA adducts in cholangiocytes. To explain carcinogenesis in human CCA, we aimed to identify and quantitate the oxysterols in liver tissue and bile from CCA patients by using gas chromatography and mass spectrometry. Focusing on the different profiles among CCA, hepatoma and cadaveric donor, we found the increased level of inflammation-related oxysterols; triol, 3K4 and 24-hydroxycholesterol (24-OH) in CCA liver tissue and bile. Moreover, higher levels of triol and 3K4 were also found in the hepatic bile compared to gallbladder bile from CCA patients, suggesting that there is the increasing production of free radical-induced pathogenic oxysterols in CCA. Furthermore, we found the low expression of CYP39A1 and CYP7A1 enzymes, which may involve in the increasing level of 24-OH in CCA patients. Thus, we proposed that pathogenic oxysterols are produced during cholangiocarcinogenesis and the accumulation of those oxysterols may involve in the development of CCA.

P08-80
Total phenolic content and *in vitro* antioxidant/prooxidant properties of leaves and fruits from *Ligustrum vulgare* L

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Ligustrum vulgare L. is a semi-evergreen woody branched bush from the family Oleaceae, widespread and used in folk medicine and pharmacy. The aim of this study was to determine total phenolic content and flavonoid concentrations of methanolic leaf and friut extract from *L. vulgare* and the effects of extracts on prooxidant/antioxidant status in HCT-116 cells. The total phenolic content of the extracts was measured spectrophotometricaly and the obtained results were 239.47 mg GA/g for leaf extract and 180.83 mg GA/g for fruit extract. The higher concentration of flavonoids was measured in leaf extract (43.78 mg Ru/g) than in leaf extract (23.68 mg Ru/g). The concentration of superoxide anion radical ($O_2^{\bullet-}$) was determined spectrophotometricaly and obtained results indicated that HCT-116 cells exhibit increasing of $O_2^{\bullet-}$ after 24 and 72 hour of treatment with both extracts in comparison with control cells. Spectrophotometric determination of nitrites (NO_2^-) was performed by using the Griess method. HCT-116 cells exhibit decreasing of NO_2^- concentration after treatment with both extracts. Determination of total glutathione also was also performed spectrophotometricaly and we observed a decreasing of total glutation content in treated cells, compared to control cells. The presence of high levels of phenolic compounds in the extracts from *L. vulgare* may have partly contributed to the observed antioxidant activities. This finding is important from a nutritional point of view, because the extracts have evidence on the potential health benefits to human due to its high antioxidant properties, and thus may be used as a dietary supplement for the prevention of cancer and other chronic diseases.

P08-81
Recycling of dysfunctional mitochondria in coenzyme Q deficiency

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Coenzyme Q_{10}(CoQ) is a unique redox lipid that transports electrons from complexes I and II to complex III in the mitochondrial respiratory chain. In addition, CoQ is a key component of the antioxidant system in all intracellular membranes, being the only endogenously synthesized lipid soluble antioxidant (Betinger M, *et al* 2007).

CoQ deficiency is a rare human genetic condition that has been associated with an increasing number of clinical phenotypes including encephalomiopathy and renal syndromes (Quinzii M, *et al*. 2011). CoQ deficiency may be associated to mutations in genes directly involved in CoQ biosynthesis (primary deficiency) or in other genes unrelated to the biosynthetic pathway as well as non genetics factors (secondary deficiencies) (Trevisson E, *et al*. 2011).

In order to elucidate how CoQ deficiency affects an organism, we have investigated the pathophysiologic processes present within fibroblasts derived from patients with this syndrome. Assays of cultured fibroblasts revealed a large group of alterations, with affection of the OX-PHOS activity, increased production of reactive oxygen species (ROS) and activation of mitochondrial permeability transition (MPT). These abnormalities triggered a mitochondrial degradation process by autophagy (mitophagy) detected by enhanced expression of autophagy genes and presence of autophagosomes containing abnormal mitochondria. Autophagy in CoQ-deficient fibroblasts affected only mitochondria but not other organelles, and was partially abolished by either antioxidants or cyclosporine treatments suggesting that both ROS and MPT participate in this process. Furthermore, prevention of autophagy in this model resulted in apoptotic cell death, suggesting a protective role of autophagy in CoQ deficient fibroblasts.

P08-82
The effect of Edaravone on pancreas antioxidant, oxidant parameteres in Valproic acid induced toxicity

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Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is a potent free radical scavenger and has the antioxidant ability to inhibit lipid peroxidation. Valproic acid (VPA) is the most widely used antiepileptic drug, however, its usefulness may be compromised due to its potential adverse effects on the gastrointestinal, neurological, hematological and reproductive systems. In this study, the effect of edaravone on pancreas antioxidant and oxidant parameters in VPA induced toxicity was investigated. Female rats were randomly divided into four groups. Group I was intact control animals. Group II was given only VPA (0.5 g/kg/day) for seven days. Group III was given only edaravone (30 mg/kg/day) for seven days. Group IV was given VPA + edaravone (in same dose and time). Edaravone and VPA were given by intraperitoneally. On the 8th day of experiment, all of the animals were fasted overnight and then sacrificed under ether anesthesia. Pancreas tissues were taken from animals and homogenized. Total protein and glutathione, lipid peroxidation levels and superoxide dismutase activity were determined in tissue homogenates. The results were evaluated statistically and discussed.

P08-83
Nrf2 in the bone marrow-derived cells is essential for the high-fat diet induced atherosclerotic plaque formation

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Atherosclerosis is the major etiology underlying myocardial infarction and stroke, and strategies for preventing atherosclerosis are urgently needed. In the context of atherosclerosis, the deletion of *Nrf2* gene, which encodes a master regulator of the oxidative stress response in mammals, reportedly attenuates atherosclerosis formation. However, the precise mechanisms of protection are largely unknown. To further clarify the role of Nrf2 in atherosclerosis *in vivo*, we performed a time course analysis of atherosclerosis development utilizing an *ApoE* knockout (KO) mouse model. The results demonstrated that oil red O stainable lesions were similar in size at 5 weeks after the initiation of an HFC (high fat and high cholesterol) diet, but were markedly attenuated in *Nrf2* and *ApoE* double KO mice (A0N0 mice) compared to those in the *ApoE* KO mice (A0N2 mice) at 12 weeks. Consistent with these results, immunohistochemical analysis revealed that Nrf2 activation is observed in late-stage atherosclerotic plaques but not in earlier lesions. RT-qPCR analysis of 12-week atherosclerotic plaques revealed that Nrf2 target genes such as *Ho-1* and *SLPI*, but not *CD36*, are expressed at significantly lower levels in A0N0 mice than in A0N2 mice, and that this change was associated with decreased expression of macrophage M1-subtype cytokines in the A0N0 mice. Furthermore, bone marrow (BM) transplantation (BMT) analysis revealed that the Nrf2 activity in the BM-derived cells contributed to lesion formation. Thus, our study has characterized the positive role of Nrf2 in the development of atherosclerosis, suggesting that Nrf2 may influence inflammatory reactions in the plaques.

P08-84
Action of the flavonoid Xanthohumol (Xn), estrogenic principle content in the hop of beer, on the modulation of ROS production levels in breast cancer cell lines

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Xn is a flavonoid with estrogenic action produced by female inflorescences of hop plants (*Humus lupulus* L.). During the brewing process, hop is used to add bitterness to the beer which makes this drink the main dietary source of Xn. As a phytoestrogen, Xn binds and activates both estrogen receptor subtypes, ERα and ERβ, although it has 3-fold higher affinity for the former. Estrogenic compounds are of great interest since they are known to promote cell growth and oxidative stress in several hormone-dependent cancers. In this work we have used the estrogen-dependent breast cancer cell line MCF7 which presents both ERα and ERβ. Our aim was to study the effects of Xn on MCF7 cell proliferation and oxidative stress. To tackle this aim we measured Xn citotoxicity, cellular ROS production, levels of oxidative damage markers and antioxidant enzyme activities and levels. Results showed that Xn has a biphasic effect on cell proliferation in a dose-dependent manner, with low concentrations inducing cell proliferation whereas high concentrations inhibit cell growth. Regarding oxidative stress, Xn high concentrations induce an increase in ROS levels. This increase of oxidative stress is consistent with a decrease in some antioxidant systems (e.g. catalase and Cu/Zn-SOD). This preliminary work suggest that Xn may modulate cell proliferation and oxidative stress in breast cancer cells and, therefore, this compound may be of interest for the development of new drugs in the pharmaceutical setting. Nevertheless, further research is necessary to reach to know the action mechanism of Xn. This project was supported by a Manuel de Oya Grant from the Centro de Información Cerveza y Salud.

P08-85
Therapeutic potential evaluation of complex combination of oxovanadium with quercetin

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Flavonoids represent a group of natural polyphenolic compounds, vegetable origin for which numerous actions have been described: anti-carcinogenic, anti-viral, anti-inflammatory, reduce the risk of cardiovascular disorders. These actions are generally associated with the ability of such compounds to neutralize free radicals, to absorb UV radiation and chelate metallic ions. Recent studies have evaluated the biological activity of the quercetine with the vanadil ion, [VO (Quer) 2EtOH] on proliferation of cells (normal and tumoral) compared with vanadil (IV) and quercetine. Toxic properties or even mutagenic of quercetine found in some older studies are controversial, newer research shows that flavonoids, including quercetine, are proving to be an-

timutagenic *in vivo* a possible explanation for these conflicting data is that flavonoids are toxic on malignant cell but are less toxic or non-toxic on normal cells. In this paper we try to demonstrate *in vivo* the biological action of oxovanadil -quercetine and highlighting the role of the concentration and the time of the administration of this compound to keep the antioxidant/pro-oxidant balance latter being responsible for the toxicity of the investigated compound . For this purpose were used as experimental animals (Wistar rats) bearing tumors (Walker carcinoma) treated three times a week with 50 mg/kg body oxovanidil-quercetine in DMSO at 7 days from the tumoral graft, for 3 weeks. We investigated biochemical parameters of serum oxidative stress (lipid peroxides, copper-oxidative activity of ceruloplasmin, albumin thiol groups and the ability of serum to reduce iron) in dynamics of treatment. The obtained results indicate an antioxidant action of compound in the first week of treatment followed by a number of side effects that led to the death of the animal along with the extension of the treatment. One can say with certainty that any compound that attend oxide-reduction metabolic reactions may come from an antioxidant to a pro-aggressive oxidizer when is outdated and time scales in the optimum necessary treatment.

P08-86
Effect of combined simvastatin/ezetimibe administration on taurine levels and oxidative status in patients CDK

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Lipid-lowering therapy has been reported to reduced several oxidative stress (OS) markers in hypercholesterolemia. Since OS is frequently associated with renal dysfunction, we aimed to investigate the effect of hypolipidemic drugs on oxidative stress and on plasma levels taurine (Tau), malondialdehyde (MDA) and allantoine /uric acid (All/UA) ratio in chronic kidney disease (CDK). Tau is a sulphur-containing amino acid that is widely distributed in animal tissue. It is involved in the catabolism of cholesterol were the latter is converted to bile acids in the liver, which are then conjugated with Tau or glycine before secretion into the bile. We enrolled 30 CDK randomized to receive three different hypolipidemic regimens for 12 months: simvastatin 40 mg/day (group 1, n = 10) or EZE/simvastatin 10/20 mg/dye (group 2, n = 10) or EZE/simvastatine 10/40 mg/dye (group 3, n = 10). The OS were evaluated at baseline and at 4, 8 and 12 months of therapy. The OS indices evaluated were significantly altered in CDK patients, moreover we found lower levels of plasma Tau in nephropathic subjects. A significant decrease of MDA and All/UA was observed during the course of therapy for all patients. Conversely, the concentration of Tau increased during the drug treatment. The mean values of Tau are inversely correlated with the mean value of MDA and All/UA for all patients ($r = -0.978$, $p = 0.002$ and $r = 0.975$, $p = 0.025$ rispectively) with a significant correlation in particular a patients of group 3 ($r = -0.992$, $p = 0.008$ and $r = 0.956$ $p = 0.044$, respectively). We hypothesize that Tau may be responsible for the oxidative stress improvement observed during lipid-lowering treatment through the reduction of superoxide anion production at the respiratory chain activity levels.

P08-87
Effect of olive leaf extract treatment on oxidative stress in liver, heart and brain tissues of aged rats

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Oxidative stress plays an important role in aging. Supplementation of several antioxidants is reported to be beneficial in reversing the deleterious effects of free radicals on aging, although some controversial data are also available. Olive tree (*Olea Europaea* L.) leaf extracts (OLE) have been used in traditional folk medicine in Mediterranean countries. OLE is rich in polyphenols and flavonoids and has antiatherogenic, antiinflammatory, hypoglycemic and hypocholesterolemic effects. All of these positive effects are partly related to its antioxidative impact. In our study, we used young (5 months) and aged (22–23 months) male Wistar rats. OLE was obtained from Yalçın Kayadibi–Ayvalık. OLE (6 ml/l in drinking water) was given for two months. Malondialdehyde (MDA), diene conjugate (DC), glutathione (GSH) levels and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) were determined in liver, heart and brain tissues of rats. According to our results, MDA and DC levels increased in liver, heart and brain of aged rats. Although GSH levels remained unchanged in heart and brain, GSH levels decreased in liver. There were no changes in SOD, GSH-Px and GST activities in respective tissues of aged rats. OLE treatment caused significant decreases in MDA and DC levels in liver and heart tissues, but not in brain tissue. Low GSH levels also increased in liver tissue following OLE treatment. This treatment did not affect tissue antioxidant enzyme activities in these tissues of aged rats. Our results indicate that OLE may be an effective agent to decrease the oxidative stress in aged rats.

P08-88
Purification and characterization of human placental glutathione *S*-transferase-pi

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Glutathione-*S*-transferases (GSTs) have a major role in the detoxification of xenobiotics, synthesis of steroids and eicosanoids, degradation of eicosanoids and aromatic amino acids, in signalling cascades and serve as ligandins for various substrate and non-substrate compounds. Cytosolic, mitochondrial and microsomal isoforms have been identified for GSTs. GST-ιπ was purified from human placenta by using following techniques: homogenization, ammonium sulfate precipitation, dialysis, affinity chromatography on *S*-hexylglutathione Sepharose 6B column and chromatofocusing on PBE94. Confirmation of the purified enzyme was made by substrate specificity experiments and molecular weight determination. The specific activity, yield and purification fold were 69.3 U/mg protein, 114% and 1321 fold, respectively. A single peak was obtained from chromatofocusing at isoelectric point of 4.8. Purifed GST-ιπ gave a single band on both native gel electrophoresis and SDS/PAGE and subunit molecular mass was determined as 24 kDa. According to substrate kinetics, K_m values for GSH and CDNB were 0.16 ± 0.04 and 3.60 ± 1.67 mM, respectively. When GSH is the varied substrate V_m was calculated as 53 ± 3 U/mg protein. V_m value of

182 ± 63 U/mg protein was obtained in conditions in which CDNB was the variable substrate. At variable GSH and variable CDNB concentrations the k_{cat} values of 7.0×10^6 and 1.42×10^7 sec^{-1} and the k_{cat}/K_m values of 4.38×10^{10} and 3.94×10^9 M/sec were obtained, respectively.

Keywords: glutathione-*S*-transferase-pi, purification, characterization

P08-89
Study of antioxidants effect at the air-water interface: a biophysical approach to membrane peroxidation

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Free radical peroxidation of unsaturated lipids in biomembranes disrupts their important structural and protective function and is related to significant pathological events. Antioxidants have a relevant role protecting membranes from peroxidation, either by the capability to scavenge free radicals, or by interacting with membranes and changing their biophysical properties in a way that favors their interaction with lipid radicals (1). Although the scavenging properties of antioxidants have been widely accessed, their biophysical role at the membrane level is most of the times overlooked. In this context, the current work aims to provide a biophysical approach of the effect of peroxyl radicals resultant from a hydrophilic generator, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), and five antioxidants: tocopherol, trolox, glutathione reduced, ascorbic acid and uric acid. Biomembranes were mimicked using lipid monolayers as membrane model systems, since this provides a convenient model for understanding bilayer structures. Furthermore, since monolayers can be studied in a Langmuir trough, where both the temperature and the mean molecular area can be controlled, we were able to extensively probe the thermodynamics of the liquid expanded to liquid condensed phase transition. Different lipid compositions characteristic of the biomembranes were used: DPPC (L-α-dipalmitoylphosphatidylcholine), DPPC:linolenic acid (9:1), EPC (L-α-phosphatidylcholine), and EPC:linolenic acid (9:1). The extensive range of peroxidation related phenomena and monolayer structural changes were analyzed both by π/A isotherms and Brewster Angle Microscopy (BAM) analysis measuring some parameters as phase transition, collapse area, collapse pressure and compressibility.

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P08-90
The endoplasmic reticulum physiology is modulated by oxygen concentration

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Oxygen is essential for life; therefore a main component for each physiological state of an organ is oxygen partial pressure. There is always a balance between oxygen supply and consumption, and while consumption is dictated by metabolic requirements, delivery is severely affected by pathological states such as cancer, diabetes, infarction, etc. An endoplasmic reticulum associated process, namely the unfolded protein response (UPR) was found to be involved in determining cell survival under hypoxic conditions. We analyzed protein synthesis rate in different cells lines and at various oxygen concentration and found that for most of cell lines protein synthesis rate decreases directly proportional with oxygen concentration. Interestingly, in a mouse lymphoma cell line the protein synthesis rate increases during hypoxia. Furthermore, we analyzed the folding pathway for two secretory model proteins (immunoglobulin M and tyrosinase) in normoxic and hypoxic conditions. For concentrations as low as 0.1% oxygen, we found no significant alterations in the folding rate for these proteins induced by hypoxia. Further studies are needed with either longer than 24 hour incubation in hypoxic conditions or incubations in anoxic conditions.

P08-91
Lipid peroxidizability as a potential trade-off of increased tissular docosahexaenoic acid concentrations in neurodegenerative processes

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Polyunsaturated fatty acids play key roles in neuron survival and nervous system homeostasis. However, it is unknown the exact role in several neurodegenerative processes, as they are also targets of oxidative damage. We have evaluated the fatty acid compositional changes in several neurodegenerative diseases and models. These work suggest that an increased docosahexanoic acid content could be a common response across several diseases. To exemplify this, we will show data in amyotrophic lateral sclerosis, characterized by a selective loss of motor neurons. The spinal cord organotypic culture under chronic excitotoxicity is a *bona fide* model of this motor neuron disease. We will show the effect of chronic excitotoxicity in the lipidomic profile of lumbar spinal cord -established by LC-QTOF-. The results show that:(i) chronic excitotoxicity leads to changes in whole lipidome and fatty acid profiles in lumbar spinal cord with increased content of the highly peroxidizable docosahexanoic acid (DHA) and both their neuroprotective and lipid peroxidation-derived products; (ii) excitotoxicity increases the expression of DHA-dependent synaptic proteins drebrin and syntaxin-3; (iii) interaction with α-tocopherol enhances polyunsaturated fatty acid neuroprotective properties associated with improved mitochondrial respiratory function; and (iv)motor neuron number loss induced by chronic excitotoxicity is dependent on fatty acid supplementation in organotypic culture. The use of NSC-34 cells -a motor neuron like cell line- revealed that glial cells are needed for the protective effect of DHA-α-tocopherol combinations in motor neurons

under excitotoxicity. Finally, the natural hydrophilic antioxidants hydroxytyrosol and 4-formyl-3-(2-oxoethyl)-2-(4-hydroxyphenyl)ethyl ester are potent neuroprotectors of those motor-neuron like cells. Globally, the results indicate that increased DHA as a response over chronic excitotoxicity in neuronal tissues could need antioxidants to potentiate its protective properties while avoiding enhanced lipid peroxidizability. Supported by ISCIII PI11/1532.

P08-92
Exposure of human pulmonary vascular smooth muscle cells to sera from patients with systemic sclerosis increases intracellular reactive oxygen species levels

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Introduction: Vascular smooth muscle cells (VSMCs) phenotype switching is believed to be a key step in the onset and progression of systemic sclerosis (SSc) occlusive vasculopathy. While VSMCs migration and proliferation appear to be largely modulated by reactive oxygen species (ROS)-mediated signal transduction pathways in atherosclerosis and ageing, a similar link in SSc vasculopathy remain to be demonstrated.
Objective: to investigate whether exposure of human pulmonary VSMCs to sera from SSc patients affects intracellular ROS levels.
Methods: Sera from 15 SSc patients with overt vasculopathy (digital ulcers and/or pulmonary arterial hypertension, PAH) and from 15 age/sex matched healthy donors (HD) were collected. Intracellular ROS levels were assessed using two different fluorescence probes, the dichlorodihydrofluorescein diacetate (H_2DCFDA), which is a general oxidative stress indicator, and the Dihydroethdium (DHE) putatively more specific for the superoxide.
Results: of 15 SSc patients enrolled, nine were affected by the diffuse and the remaining by the limited form of SSc. All patients exhibited severe Raynaud's phenomenon and digital skin ulcers and two were affected by PAH. Sera were collected before therapy with prostacicline and/or endothelin receptors inhibitors. After stimulation (t = 5 min), no significant difference in intracellular ROS levels was detected between VSMCs cultured with SSc sera and the counterpart exposed to HD sera. On the contrary, at the end of evaluation period (t = 9 hour) the intracellular ROS levels resulted significantly higher in VSMCs exposed to SSc sera as compared to VSMCs cultured with sera from HD (590.76 ± 144 RFU versus 485.85 ± 99.55 RFU respectively, p = 0.037). No difference within and between groups was detected stratifying the SSc groups for disease duration, SSc subtypes, vasculopathy severity and different probes used.
Conclusions: to our knowledge, this *ex vivo* study provides for the first time the evidence that exposure to SSc sera induces significantly differences in the intracellular redox state of human VSMCs. Whether this event is recapitulated *in vivo* in SSc and whether is really coupled with VSMCs proliferation and remodeling ultimately leading to occlusive vasculopathy need to be further evaluated. Moreover, future experiments will be designed to identify SSc sera 'boosters' (e.g. antibodies anti-VSMCs, growth factors) of intracellular ROS elevation and to clearly dissect ROS signalling pathways in VSMCs.

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P08-93
Evaluation of the inhibition kinetics of amitriptyline on rat small intestine glutathione *S*-transferase alpha

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Antidepressant drugs are frequently used in cancer patients in addition to cancer therapy. A tricyclic antidepressant amitriptyline is the highly prescribed drug for mood elevation. In cancer cells, overexpression of glutathione *S*-transferase (GST) isozymes have been reported and high cellular GST levels has been linked to anticancer drug resistance. Since there are limited studies on the effects of antidepressants on GSTs, in this study it was aimed to investigate the interaction of amitriptyline with glutathione *S*-transferase alpha (GST-α). GST-α was purified from rat small intestine by using conventional chromatographic methods. Amitriptyline inhibited GST-α in a dose dependent manner and an IC_{50} value of 8.32 mM was obtained. Amitriptyline inhibited GST-α competitively when CDNB used as fixed, GSH as the varied substrate, or vice versa. When the varied substrate was GSH the K_i value of 1.45 ± 0.20 mM was calculated. Using CDNB as the varied substrate K_i was found to be 1.57 ± 0.16 mM. These *in vitro* data show that amitriptyline, via inhibition of GSTs, may have a minor supportive role in chemotherapy by inhibiting the elimination of some cancer drugs but inhibition of GSTs can also lead to increased effect of toxic electrophiles. Further studies are needed to clarify this hypothesis. Key words: Glutathione-*S*-transferase-α, amitriptyline, GST.

P08-94
Amitriptyline and clomipramine show inhibitory effect on placental glutathione-*S*-transferase-pi

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Glutathione-*S*-transferases [GSTs, E.C.2.5.1.18] constitute a large enzyme family involving in the detoxification of xeno- and endobiotics by conjugating them with glutathione. Amitriptyline and clomipramine are in the group of drugs called tricyclic antidepressants (TCA) and are widely used in depression therapy of the pregnant woman. Since the ability of these drugs to pass through the placenta has been shown, the effect of the accumulation of amitriptyline and clomipramine on fetus needs to be clarified. In this study, it was aimed to investigate the interaction of GST-ιπ with amitriptyline and clomipramine.

At fixed [CDNB] and variable [GSH], linear mixed-type inhibition was observed with amitriptyline (K_s = 0.16 ± 0.03 mM; α = 2.08 and K_i = 1.75 ± 0.37 mM) and clomipramine (K_s = 0.24 ± 0.05 mM; α = 1.57 and K_i = 3.90 ± 2.26 mM). When the varied substrate was CDNB, amitriptyline (K_i = 4.90 ± 0.68 mM) and clomipramine (K_i = 3.37 ± 0.39 mM) showed noncompetitive inhibition. Inhibitory behavior of amitriptyline and

clomipramine destroy the enzymatic barrier formed by GST-ιπ, which may further cause an increase in the exposure time of the fetus to toxic electrophiles. Due to immature hepatic clearence system of the baby, congenital malformations might be observed.
Keywords: glutathione-*S*-transferases-pi, amitriptyline, clomipramine

P08-95
Properties of the thioredoxin system in microaerophiles from the *Streptococcus genus*

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The thioredoxin system, involved in the preservation of the reduced state of citoplasmic proteins, is composed of two enzymes, thioredoxin (TrxA) and thioredoxin reductase (TrxB). To investigate on the properties of this system in the *Streptococcus* genus, the strains *S. mutans*, a pathogen involved in the development of dental caries, and *S. thermophilus*, a non pathogenic species employed in food industry, were selected. These fermenting facultative anaerobes are aerotolerant, as they possess superoxide dismutase, the first key enzyme involved in the control of reactive oxygen species; on the other hand, *S. mutans* and *S. thermophilus* lack catalase. The redundant putative genes encoding TrxA and TrxB in the genome of *S. mutans* and *S. thermophilus* were studied. Their predicted amino acid sequence was analysed to exclude products without the typical CXXC consensun. One TrxB gene in each strain, whereas two and three genes for TrxA remained in *S. mutans* and *S. thermophilus*, respectively. The TrxB gene from these strains was cloned and expressed, together with one TrxA gene from each strain. The activity of the recombinant enzymes was tested. Each TrxB catalysed the NADPH-dependent reduction of dithiobis-nitrobenzoate, and each TrxA induced the insulin precipitation in the presence of dithiothreitol. When the combined activity of the homologous enzymes was tested in the presence of NADPH as electron donor and human insulin as the TrxA substrate, only the reconstitued *S. thermophilus* system was active, thus demonstrating the direct functional interaction between the two homologous components. For the reconstitution of the *S. mutans* system, the other putative TrxA genes from this strain will be analysed. The study of the molecular and biochemical properties of the recombinant enzymes is in progress.

P08-96
The effects of neuropeptide Y on oxidative/antioxidative status in trophoblasts

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Preeclampsia is pregnancy-specific disorder of unknown etiology. Clinically is characterized by new development of hypertension ($>140/90$ mm Hg) and proteinuria. Inadequate trophoblast invasion of the maternal uterine spiral arteries results in poor placental perfusion, which leads to placental hypoxia/reoxygenation. This could results in development of oxidative stress that contributes endothelial dysfunction and clinical manifestation of preeclampsia. Neuropeptide Y (NPY) is sympathetic co-transmitter released during stress and is involved in several physiological processes such as food intake, angiogenesis and vasoconstriction. Literature data show that the levels of NPY are signifiantly elevated in preeclampsia compared to healthy pregnancy. The objective of this study was to determine the effects of NPY on oxidative/antioxidative status in trophoblasts. Cells were cultivated for 24 hour in cell culture medium which contained increasing concentrations of NPY (10^{-9}, $10^{-8}$and 10^{-7} M). Intracellular concentrations of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), nitrite (NO_2^-), reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured. Results of this study show that the concentrations of H_2O_2 and GSH were significantly increased, while the concentrations of NO_2^- were decreased in dose-dependent treatment of NPY. The highest used dose of NPY (10^{-7} M) increases the concentrations of GSSG and O_2^-. These data show that NPY induces oxidative stress in trophoblasts and suggest that it could be one of the factors involved in inadequate trophoblast invasion and consequently in preeclampsia pathogenesis.

P08-97
Melatonin reverses the harmful effects of the chemical stimulus on mast cells. Therapeutic implications

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Melatonin (*N*-acetyl-5-methoxytryptamine) is a free radical scavenger and has cytoprotective effects in inflammatory processes. Its synthesis has been found in different sites of the organism, and a major source of extra-pineal melatonin is the immune system and specifically mast cells. In this regard, RBL-2H3 cells are considered as a good tool for studying the effect of chemical stimuli (PMACI) on the activity, degranulation and release of mediators by mast cells. The results showed that PMACI incubation introduced mast cell in a state of hyper-stimulation and hiper-function, reducing the cell viability in a time-dependent manner. On the other hand, melatonin treatment at physiological and pharmacological doses 100 nM–1 mM, increased the cell viability, however only the pharmacological doses of 1 mM did so in a way significant statistically compared to cells stimulated and not treated. When a non-specific melatonin receptor antagonist, luzindole 10 μM was co-incubated with 1 mM melatonin, the cytoprotective effects of melatonin on these cells was significantly reduced. It appears that the effect of melatonin on mast cells is mediated via melatonin membrane receptors, although not entirely. In conclusion, melatonin treatment reduced the cytotoxicity, mediated by PMACI, on mast cells in culture and could provide a useful therapeutic option in allergic inflammatory disorders.

P08-98
VEGF-D modulates redox homeostasis of human endothelial cells

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Vascular endothelial growth factor-D (VEGF-D), due to regulation of lymphanogenesis and inducing angiogenesis, plays an important role in physiology of cardiovascular system. In this report we attempted to evaluate whether VEGF-D is implicated in regulation of the redox balance in endothelial cells, known to influence angiogenic signaling pathways and vascular integrity. In preliminary experiments we performed proteome analysis of HUVECs treated with VEGF-D.

Upregulation of both antioxidants (SOD2, catalase, peroxiredoxins 3 and 6) and enzymes responsible for ROS production (NADH-quinone oxidoreductase) was confirmed by Western immunobloting. VEGF-D treated HUVEC showed considerably higher production of ROS and RNS measured using fluorogenic probes, H2DCF DA and DAF-FM DA, respectively. Elevated oxidation of H2DCF and DAF was found already after 8h of treatment with VEGF-D and the highest increase in DAF oxidation amounting 150% of the control was observed after 24 hour treatment of HUVEC's with 1 μg/ml growth factor. Nevertheless thiol group content, assessed by monobromobimane oxidation, was not decreased.

To summarize, the obtained data suggest a stimulatory activity of VEGF-D on the antioxidant system in HUVECs, demonstrated by moderate stimulation of ROS/RNS production resulting from affecting the balance between antioxidant and oxidant enzymes.

P08-99
The effect of melatonin on CaMKII protein levels in glioma cells at oxidative stress condition

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Introduction: Melatonin is a hormone produced by the pineal gland in a rhythmical pattern. Melatonin has neurobiological actions mediated through cell membrane receptors and by intracellular signaling cascades. Melatonin has a possible antidepressant effect probably based on its effect on the central circadian regulation and an effect on improving cognitive function. In addition, melatonin might scavenge free oxygen radicals and thereby act as neuroprotective agent. Neuronal Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) is a ubiquitous target of CaM. CaMKII is essential for diverse physiological processes such as Ca^{2+} dependent long-term potentiation in the brain. CaMK II regulates many cell functions in response to intracellular Ca^{2+} release. Ca^{2+} homeostasis in these and other regions of the body are adversely affected by oxidative stress, due to ischemia, disease, or aging. In this study, we investigated the modulatory effects of melatonin, on glial CaMK II levels eiher in oxidative stress and non oxidative stress conditions.

Method: We used C6 Glioma cells in the study. Cells were devided into 75 cm^2 cell flask, the last volume was 10 ml in each flask. Culture flasks was marked into four groups which one of them was control group, the others were treated with H_2O_2 (5×10^{-4}), melatonin (5×10^{-4}), and melatonin+ H_2O_2. All plates were incubated for 72 hours at 37°C in incubator. After incubation, protein extraction was performed with Trizol and Western Blotting was perfomed for the evaluation of CaMK II protein expression. We used Image J programme for dansitometric analyze and Graph Pad Prism for statistical data analyze.

Results: We found increased CaMK II protein levels after H_2O_2 administration in glial cells compared with control group. This increased in CaMK II protein level was significantly lower in the presence of melatonin (5×10^{-4}).We found no clear differences between melatonin administrated group and control group.

Our preliminary results show that melatonin could change the calcium homeostasis and these changes may play a role on its neuroprotective effect.

P08-100
Cellular targets of the novel type of neuroprotective 1,4-dihydropyridine derivative

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Background: Our previous data have shown the neuroprotective activity of several 1,4-dihydropyridine derivatives (Vicente *et al*., 2006; Klimaviciusa *et al*., 2012). The aim of our present study was to investigate the possible neuroprotective activity and its cellular targets of the novel type of 1,4-dihydropyridine (DHP) derivative compound CB-56.

Methods: Neuroprotective activity of CB-56 was tested in cytoskeletal disrupting agent colchicine-induced toxicity model in rat primary cortical neurons. Cell death was assessed by Trypan blue method. The effect of CB-56 on the cell viability was studied in HEK-293 and SH-SY5Y cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and lactate dehydrogenase (LDH) method only in HEK-293 cells. The free radical scavenging activity was determined by using diphenylpicryl-hydrazyl (DPPH) method.

Results: CB-56 decreased colchicine-induced cell death in rat primary cortical neurons at the concentration of 10 μM by approximately $40^0/_{00}$. Tested compound did not effect cell viability added per se neither in rat primary cortical neurons, nor in HEK-293 and SH-SY5Y cells. CB-56 did not increase the LDH release in HEK-293 cells. The obtained results showed, that tested compound exerted the free radical scavenging activity in DPPH test at the 10 μM concentration.

Conclusions. The novel 1,4-dihydropyridine derivative CB-56 protected rat primary cortical neurons against colchicine-induced cell death and did not induce cytotoxicity in HEK-293 and SH-SY5Y cell lines and rat primary cortical neurons. This neuroprotective effect could be partly explained by the free radical scavenging activity. Therefore, CB-56 could be regarded as a prototype molecule for the design of novel neuroprotective drugs.

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P08-101
Role of ade8 mutation in stress resistance of yeast Saccharomyces cerevisiae CEN.PK strain backgrond

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Gene Ade8 is commonly used gene integration place (Sadowski, 2007) We have shown that deletion of this gene in yeast CEN.PK genetic background gives increased oxidative stress, desiccation and heat shock resistance. Salt and osmotic stress tolerance is not influenced by ade8 deletion. Increased desiccation tolerance, accompanied by elevated accumulation of trehalose, is observed only in adenine limitation conditions, whereas oxidative stress resistance is independent of adenine limitation. These changes in stress resistances were not observed if the deletion of ade8 was made in S288C genetic background (strain BY4741). Possible causes of differences among strains in their genetic background will be explored. Possible role of cyr1 or trp1 mutation in resulting differences between strains and their stress resistances will be examined.

P09 – Genomics and Proteomics

P09-1
Modeling of 3-dimensional structures of melanocortin receptors

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Melanocortin receptors (MCRs) are seven-transmembrane domain proteins that belong to the rhodopsin family of GPCRs. There are five known MCR types, MC1-5R, and they are promising targets for novel drugs. However, design of drugs for them is hampered because of the lack of accurate 3D-dimensional structures for these receptors. This study was therefore devoted to modelling of the 3D-structures of the human MC1-5Rs, with the aim to obtain models good enough for use in drug design.

Sequence alignments were done with the ClustalW algorithm http://www.uniprot.org/), which showed that the human MCRs have the highest homology with the human adenosine A2A receptor among the GPCRs for which X-ray crystal structures are available. The X-ray structure (PDB ID 2YDV) of the A2A receptor was accordingly chosen as template for the modelling. Detection of start and end positions for the helices was done using resources for protein secondary structure prediction on the PSIPRED Server and the ALB program, with subsequent analysis of conservative regions. The 3D structures of the MC1-5Rs where built using MODELLER9.9.

Analysis of the 3D models showed that the ligand-binding cavity of the MC3R, by contrast to the other MCRs, has a large positively charged region. Moreover, the MC1R showed the smallest ligand-binding cavity (~190 Å3), while the MC4R binding cavity was the largest, being more than 2-fold larger (~450 Å3) compared with the MC1R; the volumes of the MC3 and MC5R cavities fell in between (~260 Å3). These differences seem to correlate with the different sizes of the preferential natural ligands for the MCRs.

The 3D models are now in use for docking with natural and artificial ligands, with promising results, which will be reported.

P09-2
Rapid analysis of protein tyrosine sulfation/desulfation

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Protein tyrosine sulfation is a post-translational modification (PTM) that regulates protein-protein interactions and, in turn, influences biological functions, such as HIV entry, enterovirus 71 infection, inflammation, blood coagulation, and sterility. The sulfated proteins can be further regulated through desulfation catalyzed by sulfatase (EC 3.1.6.). Desulfation links to many important physiological functions, including cellular degradation, hormone and signaling pathways regulation. Sulfatases are known to involve in various pathophysiological mechanisms, such as lysosomal storage disorders, hormone-dependent cancers, developmental abnormalities, and bacterial pathogenesis. In this report, we described the use of enzyme-linked immunesorbent assay (ELISA) platform for the analysis of protein tyrosine sulfation and its following desulfation. Sulfated protein was prepared through a protein sulfation platform that includes 3′-phosphoadenosine 5′-phosphosulfate synthetase (PAPSS) and tyrosylproteinsulfotransferase (TPST) coupled reactions. Desulfation of the sulfated protein was catalyzed by sulfatase. Glutathione *S*-transferase and P-selectin glycoprotein ligand-1 fusion protein (GST-PSGL-1) was used as model protein for this study. Our results indicated that the degree of GST-PSGL-1 sulfation can be rapidly determined using a 96-well microreader plate. The activities and selectivity of TPST and sulfatase toward the sulfation and desulfation of GST-PSGL-1 were determined. Together with other methods, such as site-directed mutagenesis, the mechanisms of protein sulfation/desulfation were investigated.

P09-3
Chaperones suppress the toxicity of aberrant protein aggregates. Molecular insight into the mechanism of action

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Deficiencies in the homeostasis of the proteome lead to many human pathologies. Chaperones play a key role in the regulation of proteostasis and are known to facilitate folding, inhibit aggregation and promote clearance of misfolded aggregates. Little is known, however, about their ability to suppress the toxicity of aberrant protein oligomers, which are considered the major deleterious species in protein misfolding diseases. The effect of five human chaperones, namely αB-crystallin, Hsp70, clusterin, α_2-macroglobulin and haptoglobin, was tested on the toxicity of misfolded oligomers. Measures of cell viability showed all five chaperones are effective in suppressing the damage caused by these protein aggregates. An investigation at the molecular level showed that the ThT-binding of the oligomers was not affected by chaperones, indicating that they do not dissolve the oligomers. Confocal microscopy, intrinsic fluorescence, SDS/PAGE and immuno-dot blot, revealed the chaperones bind to the oligomers. Atomic force microscopy images showed that, following binding, the chaperones induce the assembly of the oligomers into larger species. In these complexes the molecular structure of the aggregates is preserved as indicated by infrared spectroscopy and site-directed labeling experiments using pyrene. These data suggest that the chaperones bind and induce the clustering of the oligomers, reducing the exposure of their reactive surfaces and decreasing their diffusional mobility. For these reasons, the clusters are unable to interact with cell membranes, which are protected from damage.

P09-4
Proteomic characterization and pharmacological activities of the venom of the Moroccan cobra Naja haje legionis

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In this work we report the first proteomic and pharmacologic characterization of the venom of the Moroccan cobra Naja haje legionis. For this purpose we used a combination of proteomic techniques such as gel filtration, RP- HPLC, 1D electrophoresis, in gel digestion, tandem Mass spectrometry and protein database search. We also report the pharmacological characterization of this venom using *in vitro* preparations from rodents and chicks. This approach allowed the identification of 106 protein and peptide from database that can be classified in 17 protein families according to their biological activities. We report the identification of: cobra venom factor, l-amino-acid oxidases, acetylcholinesterase, metalloproteinase, disintegrin, cysteine rich secretory proteins, nerve growth factor, phospholipases A2, vespryns, kunitz-type inhibitor, short neurotoxins, long neurotoxins, Weak neurotoxins, neurotoxin like proteins, muscarinic toxins, cytotoxins and cardiotoxins. The presence of these activities was confirmed by pharmacology tests that shows that the venom of Naja haje legionis induce irreversible blockage of neuromuscular transmission in both rodent and chick preparations and exhibit neurotoxic, myotoxic and cardiotoxic activities. The contribution of these proteins and peptide in the toxicity of Naja haje legionis venom deserves farther investigations.

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P09-5
Proteome of human blood lymphocytes exposed to multiwall carbon nanotubes

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To examine human toxity of multiwall carbon nanotubes (MWCN) we used proteomic approach to evaluate alterations in the protein expression in primary culture of lymphocytes. Human T lymphocytes for cell cultivation were isolated from blood of healthy donors. We found that total protein value in lymphocytes exposed to MWCN was significantly decreased as compared with control cells (without MWCN treatment). To investigate lymphocyte proteome composition the 2D-proteomic maps were obtained. MWCN were found to cause considerable changes in proteome profile of lymphocytes. Some proteins of interest were excised from the gel, digested and identified using MALDI-TOF mass spectrometry. Identification was successful for 10 proteins. Analysis of their functions showed them to participate in cellular defense response against oxidative stress (glutathione transferase), nucleic acid structure and metabolic processes (mismatch repair protein, polymerase isoforms, single-stranged DNA-binding protein), protein synthesis and function regulation (polymerase isoforms, protein phosphatase isoforms), carbohydrate balance regulation (glycosyltransferases isoforms).

P09-6
Lectin based proteomic profiling of human and ruminant milk fat globule membrane proteins

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The protective coat that surrounds the lipid globules in milk is known as the milk fat globule membrane (MFGM). MFGM harbors a considerable number of proteins, which comprise 4% of the total protein content in milk. The functions of some of the MFGM proteins have yet to be deciphered. Identification of the proteins found in MFGM is highly informative and may reveal unknown interactions or pathways that occur in the mammary glands. Recent studies have reported numerous bioactive proteins in MFGM that play significant roles in cell signaling and defence mechanisms. The present study aims to compare the MFGM proteome profiles of ruminants and humans as well as to analyze proteins that may be glycosylated. MFGM protein extracts were subjected to two-dimensional gel electrophoresis (2DGE) and subsequently developed using silver staining or transferred onto nitrocellulose membranes for lectin blotting. The respective 2DGE profiles and lectin blots were analysed using Image master 2D Platinum software 7 and the resolved proteins were identified using mass spectrometry and database search. The MFGM proteins that were identified in this study are suggestive of the different interactions or pathways that occur in the mammary glands of ruminants and humans.

P09-7
Analysis of sera of patients with osteosarcoma using 2-dimensional electrophoresis-based proteomics

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Bone tumour refers to a neoplastic growth which originates from various types of bone tissues, the surrounding soft tissues, muscles and ligaments. Currently, biopsy is the only option available in order to confirm whether the tumour is really a cancer and not some other types of bone diseases. Therefore, it is imperative to find a less invasive way to diagnose the disease. In this study, the expression of serum proteins was analysed using the proteomic approach. The method has been widely utilised by researchers to elucidate biomarkers from numerous types of cancers. We focused on osteosarcoma, also called osteogenic sarcoma, which is the most common type of primary bone tumour. Two-dimensional electrophoresis was carried out to profile proteins from sera of newly diagnosed patients with osteosarcoma and age-matched normal healthy individuals. The data were analysed using Image Master Platinum version 7.0 and protein spots with different altered expression were detected compared to the controls. The aberrantly expressed serum proteins identified in this study offer tremendous potential for use as biomarkers to aid early diagnosis of osteosarcoma.

P09-8
Nucleoprotein conjugates for the identification of protein biomarkers of pathological states

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The discovery and identification of low-copy-number protein and peptide biomarkers of pathological states is one of the fundamental goals of current biomedicine and practical proteomics. The progress in the methods of polypeptide identification is caused by developments of instrumental methods of analysis and of methods of affinity-based concentration of analytes. The project goal is the investigation of means to improve biomarkers detection limits and evaluation of feasibility of application for that purpose DNA-based artificial antibodies (DNA aptamers). Such synthetic aptamers have active groups able to form covalent bonds with target molecules following the formation of affinity complexes. Fixing of biomarkers as nucleoprotein conjugates makes them accessible for the identification by various methods, for example, PCR amplification. In current work optimal conditions of photo induced reactions for obtaining the conjugates of peptide of sequence DRVYIHPF with oligodeoxyribonucleotide bearing 5-Br-U residues was found. Optimized instrumental methods (HPLC and MALDI-TOF MS) for robust control of photochemical reactions Was found optimal conditions of selective hydrolysis of conjugates (up to peptide attachment place) and of simultaneous exhaustive cleavage of free oligomers.

The system of Real-Time PCR amplification for quantitation of nucleopeptide conjugates content in complex mixtures was created. With its use, the reliable quantitation of $\sim$ 60 molecules of peptide DRVYIHPF was demonstrated.

Obtained results are important not only for the development of modified DNA generation methods, but structural studies on protein-nucleotide interactions and directed creation of nucleotide-based medicine.

P09-9
Cytokine genes expression profile and their association woth essential hypertension

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Essential hypertension (EH) is a common disease with severe cardiovascular complications. Inflammation and endothelial dysfunction are important factors contributing to blood pressure elevation. Experimental studies have demonstrated up-regulation of pro-inflammatory cytokines expression in circulating leukocytes of hypertensive patients. The aim of the present study was to evaluate cytokine genes expression profile in peripheral blood cells of patients with EH and to investigate the association between EH and the polymorphisms in genes with altered transcriptional activity. RNA and DNA was isolated from peripheral venous blood of 364 patients with EH and 273 control subjects. 84 cytokines genes were screened for their expression profile with RT^{2}Profiler^{TM} PCR Array (SuperArray Bioscience Corporation, USA). Genotyping was performed using PCR and PCR-RFLP methods. The expression profiling has shown altered transcriptional activity of *CCL16*, *CCL17*, *CCL18*, *CCL19*, *CCL23*, *CCL8*, *CCR6*, *CCR8*, *CX3CR1*, *CXCL1*, *CXCL13*, *ICEBERG*, *IL13*, *IL17C*, *IL1F10*, *IL1F6*, *ILF9*, *SPP1*, *CD40LG*, *XCR1* and *CCL2* genes in patients with EH. Further analysis of individual gene expression confirmed up-regulation of *CD40LG* and down-regulation of *CCL2* genes. Moreover, we found that *CD40LG* and *CCL2* genes expression levels correlated with systolic blood pressure levels. Haplotype-based analysis of *CCL2* gene (rs1860190, rs1024611, rs3917887, rs991804) showed that *CCL2**AGDT haplotype was associated with the increased risk of EH (OR = 1.53, p = 0.0099). Logistic regression with subsequential multifactor dimensionality reduction were used to investigate interactions between studied SNPs. *SELE**C/T genotype and *CXCR3**C alelle combination showed the most significant association with EH (OR = 1.73, p < 0.001).

P09r-10
Functional metagenomics: enrichment culture versus direct isolation. A practical case

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Metagenomics involves the study of the total DNA of a microorganisms community as a whole, called metagenome, allowing the analysis of unculturable microorganisms, which comprise more than the 99% of the natural microbial diversity. In functional metagenomics the goal is the discovery of new enzymes. Total DNA is extracted from environmental samples and used for the construction of metagenomic libraries and then the clones are screened for the enzymatic activity of interest.

There are several strategies to raise the frequency of positive clones. An enrichment culture, where the growing of the microorganisms carrying the enzyme of interest is favoured by a selective pressure, is an example. The main disadvantage of enrichment cultures is the dramatical decrease of microbial diversity given that only the culturable microorganisms can be present.

In this work we analyze the results of the screening for lipolytic activity of two metagenomic libraries in *Escherichia coli* with the fosmid pCC1FOS, constructed from environmental samples of a thermal springs following two strategies: direct isolation of metagenomic DNA and isolation of DNA from enrichment culture with olive oil as the only source of carbon. We evaluated not only the frequency of positive clones, but also the redundancy and the novelty of the enzymes detected in the screening.

P09-11
Expression of haptoglobin in human colorectal cancer

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Haptoglobin (Hp) is an acute-phase glycoprotein, a heterotetramer that consists of two alpha and two beta chains. The latter contain N-linked oligosaccharides characterized by terminal alpha(2-6)-linked sialic acid residues. Hp is mainly known for binding hemoglobin to prevent iron loss and kidney damage during hemolysis. CDw75 is a sialylated epitope whose expression seems to be related to colorectal cancer (CRC) malignization process. In a recent study our research group found Hp as a CDw75-carrying glycoprotein in CRC. Although Hp has been suggested to be expressed in colorectal tissue, not a single deep study of its expression in this tissue has been published to date.

Here, we analyze Hp expression in normal and tumoral tissue surgical specimens from CRC patients. Briefly, we performed an immunohistochemistry analysis of Hp expression in formalin-fixed and paraffin-embedded sections from 12 normal and 56 tumoral specimens of CRC. To this purpose we used a monoclonal antibody (Abcam ab13429) to detect Hp and EnVision™ HRP detection system (Dako) for the developing. Evaluation of the slides was carried out by an expert pathologist. The results indicate Hp is not expressed either in the normal specimens or in the non-tumoral areas of tumoral ones. Concerning the tumoral specimens, 9 out of the 56 (16%) showed Hp expression. When present, Hp expression is almost always weak and focal, restricted to epithelial cells of colorectal glands. Results in our hands show that Hp is present in tumoral mucosa but not in normal colorectal tissue. However, the limited distribution and intensity of pattern immunostaining suggests that Hp is not a good candidate to become either a diagnostic or a prognostic marker for CRC malignancy.

P09-12
Comparative proteomics of CD133+ and CD133− colon cancer cells reveals activation of the Wnt pathway and a potential therapeutic role of SRp20

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Recent studies revealed a sub-population of cancer cells called 'cancer stem cells' (CSCs) that eludes treatment and regenerates cancer. In the attempt to shed light on the molecular mechanisms typical of CSCs, we studied colon CSCs using two colon cancer cell lines: $CaCo_2$ and HCT-116. Putative CSCs were separated from non-CSCs by flow cytometry using CD133 as stemness marker. Then, protein extracts of CD133+ cells were compared to protein extracts of CD133- cells. Differentially expressed proteins were identified by 2D-DIGE coupled with tandem mass spectrometry. We found 49 differentially expressed proteins in CaCo-2 CD133+ versus CD133- cells and 36 in HCT-116 CD133+ versus CD133- cells. Ingenuity Pathway Analysis of the differentially expressed proteins revealed an alteration of energy metabolism. Examination of the resulting network showed that several proteins were connected to the Mutated in Colorectal Cancer (MCC) gene, i.e., a negative regulator of the Wnt pathway. Among the proteins identified, we found a 2-fold change up-regulation of the splicing factor SRp20, which is a newly identified target gene of the Wnt/β-catenin pathway. Moreover, we found a direct cause-effect relationship between Wnt pathway activation and the SRp20 expression. We also showed that SRp20 influences cell proliferation thereby implicating SRp20 in the tumorigenicity of CD133+ cells. Our results demonstrate that when SRp20 is silenced, MCC expression is increased, while β-catenin and γ-catenin expression is decreased, which suggests a slowing-down of the Wnt pathway. In conclusion, we found a positive correlation among CD133 expression, Wnt pathway activation and increased SRp20 expression, and raise the possibility that SRp20 modulates the Wnt pathway by affecting MCC expression.

P09-13
Integrating omic technologies into environmental risk assessment

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Integration of genomic-based methodologies into ecotoxicology has permitted to maximize the information obtained from limited testing species, to identify the mechanisms of toxicity of existing and emerging pollutants, to link molecular and cellular biomarkers with higher level population and ecosystem responses, and to anticipate potential ecological risks. We have used transcriptomic, proteomic and metallomic methodologies to study different zones in Southwest Spain, mainly Huelva city and the boundaries of the Doñana National Park, one of the most important European biological reserves, affected by industrial and intensive agriculture practices that generate a big amount of pollutants, reaching the Park and its inhabitants. *Mus spretus*, an unprotected mouse that typically inhabits marshlands, and red swamp crayfish *Procambarus clarkii*, one of the most important freshwater decapods farmed for consumption, have been used as sentinel organisms. It is well established that both are excellent bioindicators for assessing the effects of contaminants in terrestrial and aquatic environments, respectively. The difficulties of working with non-model organisms as bioindicators have been solved by combining several *omic* approaches. Our results with heterologous microarrays in *M. spretus* and suppressive subtractive hybridization (SSH) in *P. clarkii* indicated that animals sustaining a heavy pollution burden exhibited an enhanced immune, oxidative and xenobiotic stress responses and/or cell apoptosis. The proteomic studies provided a holistic insight regarding the manner by which pollution shifts protein amounts in two-dimensional gel electrophoresis, and the peptide amounts in iTRAQ. The metallomic analysis revealed the presence of low molecular mass metallothionein-like proteins in animals captured at polluted areas, according the high metal levels found in these animals tissues. This integration of metallomics with proteomics and transcriptomics can be useful in further studies for assessment of environmental issues. (Grants: CTM2009-12858-C02-02 and CVI-3829).

P09-14
The type VI secretion systems encoded in Salmonella pathogenicity islands SPI-6 and SPI-19 contribute to intestinal colonization of chickens and systemic spread by *Salmonella enterica* serotype Typhimurium

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The Type VI Secretion System (T6SS) is a recently described protein secretion system in Gram negative bacteria. *Salmonella enterica* contains four phylogenetically-distinct T6SSs encoded in differentially-distributed genomic islands. *S. enterica* serotype

Typhimurium harbors a T6SS encoded in SPI-6, while serotype Gallinarum harbors a T6SS encoded in SPI-19. Both SPI-6 and SPI-19 encode each of the core components described for T6SSs; however, they differ in terms of genetic organization, sequence identity and phylogenetic origin.

S. Gallinarum and *S*. Typhimurium are able to infect chickens but with different outcomes. *S*. Gallinarum is a pathogen restricted to poultry where it causes a severe systemic disease, whereas *S*. Typhimurium is a broad host range pathogen that colonizes poultry sub-clinically. We have reported that SPI-19 contributes to systemic spread of *S*. Gallinarum in chickens. Here we investigate the role of SPI-6 in infection by *S*. Typhimurium. Also, we have addressed whether SPI-6 and SPI-19 are functionally interchangeable or contribute to the differences in infection of the avian host.

White Leghorn chickens were inoculated orally with a mixture 1:1 of the wild type (WT) and a mutant carrying a non-polar deletion of SPI-6 (SPI-6). At days 1, 3 and 9 the chicks were sacrificed, the liver, spleen, ileum and ceca were recovered and the bacterial loads were determined. Results showed that the SPI-6 mutant colonized all the organs significantly less than the WT. Introduction of the intact island into the mutant strain restored bacterial colonization of internal organs. Transfer of SPI-19 from *S*. Gallinarum to the SPI-6 mutant of *S*. Typhimurium not only complemented the defect but also resulted in a transient increase in ceca and ileum colonization at days 1 and 3. At day 9, similar levels of bacterial loads for the mutant complemented with either SPI-6 or SPI-19 were recovered.

Our data indicate that both SPI-6 and SPI-19 contribute to chicken colonization and that SPI-19 confers an additional advantage in early colonization of the gastrointestinal tract of infected chickens. This work was supported by grant 1100092 from FONDECYT.

P09-15
Opposite effects of myostatin and follistatin on transcriptomic profile of differentiating C2C12 mouse myoblasts

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GDF8 (myostatin) is one of the strongest inhibitors of muscle development. FS (follistatin) is an activin-binding glycoprotein, known for its antagonistic function on the TGF-beta super family of cytokines, including myostatin. The influence of GDF8 (20 nM) and FS288 (6.5 nM) on C2C12 myoblasts differentiation was evaluated using immunofluorescence analysis of MyHC protein expression and by comparative analysis of the number and type of myotubes. Additionally microarray technique was used for a comprehensive study of changes in the transcriptional profile of differentiating C2C12 mouse myoblasts treated with GDF8 and FS. The gene expression was evaluated using the Agilent whole mouse genome microarrays and validated with Real Time PCR method. The observed differences are the number and size of myotubes clearly demonstrated the inhibitory effect of GDF8 and stimulatory influence of FS on myoblasts differentiation. The statistical analysis of the results of transcriptomic profiles revealed 155 contrary regulated genes. Based on ontological analysis we concluded that myostatin-related effect was mainly mediated by regulation of 39 development regulatory genes (e.g. Hgf, Fgf2, *Myf6, Cdc42, Sort1*), 23 regulators of transcriptional processes (e.g. *Stat1, Lef1, Lmo1*), 13 calcium binding proteins (e.g. *Trdn, Mmp3, Nkd1*) and influence on focal adhesion (e.g. *Itga-6, Itgb-1,3, Tmod1, Tubd1*). The obtained results showed that myostatin-related effect could be mainly mediated by regulation of transcription, modulation of cell adhesion, regulation of calcium metabolism and changes in expression of genes connected with developmental processes. We can hypothesize that the GDF8 is able to exert also a secondary effect on differentiating C2C12 myoblasts through the regulation of Mef2c, Edn1 and Fst expression.

P09-16
Cytokines profile expression in pancreatic cancer patients

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Pancreatic ductal adenocarcinoma (PDCA) is one of the most aggressive human malignancies. Until today, all available treatment modalities, such as chemotherapy and radiotherapy have had little impact on the clinical course of the disease. Thus, a better understanding of the molecular biology of PDCA is important to develop potential and targeted therapies and to establish novel detection markers. Interactions between the immune system and tumor cells have been thought to play a pivotal role in tumor progression. In particular, in PDCA, microenvironment, with its distinct inflammatory reaction, plays an active role in its development. Recent findings have demonstrated that tumor cells themselves produce cytokines and chemokines, thereby leading to a modulation of the microenvironment, playing an important role in the regulation of angiogenesis, growth, invasiveness and metastasis of tumor. We have performed a novel cytokine antibody arrays from RayBiotech (RayBio® Human Cytokine Array) as a method to identify 507 cytokines in serum of PDCA patients and controls. All patients were diagnosed of PDCA and were treated under combined treatment *Gemcitabina + Erlotinib*. Samples of blood were taken before and after treatment. Final spot intensities were measured as the original intensities subtracting the background. Data were normalized to the positive controls in the individual slide. Quality analysis was performed using the '*ArrayQualityMetrics*' package in *R* to get rid of any feasible outlier. We carried out two types of comparisons. First, we analyzed patients pre-treatment against controls. Secondly, we analyzed patients pre-treatment against patients post-treatment. We chose those proteins that were common in both analyses to avoid the side effects of the drugs. Differences between groups were analyzed for significance with an ANOVA test ($p < 0.05$). Besides, fold change (FC) was calculated. Any ≥1.5-FC or ≤0.65-FC in signal intensity between groups was considered relevant. Using this technology, 6 proteins were found to be useful as diagnosis or as prognosis markers.

P09-17
Identification of biomarkers in diabetes mellitus patients by serum proteomic analysis (DIGE)

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Over the last 10 years, the prevalence of diabetes has rapidly and globally increased reaching epidemic proportions. Diabetes is linked to a number of complications, being the most prominent cardiovascular disease. A diagnosis of diabetes at an early stage is therefore essential to reduce the burden of this disease and its complications on individuals. Pathological changes can be reflected in serum proteomic modifications. Thus, biomarkers discovered in serum may form the basis of non invasive and simple diagnostic tests or be candidates for therapeutic intervention. We have performed a proteomic study including 18 males divided in three different groups: (i) six subjects with DM2 and cardiovascular complications; (ii) six subjects with DM2 without cardiovascular disease; (iii) six healthy donors as control. One of the major difficulties in the identification of biomarkers is due to the fact that these proteins are minority in serum or plasma. Albumin, a2-macroglobulin, transferrin, and immunoglobulins, may represent about 80% of total serum protein. These proteins provide a significant background and make it difficult to identify low abundance proteins in serum using the traditional 2D Electrophoresis approach. To overcome this problem all serum samples were depleted of the 17 most abundant serum proteins. Protein profiling was separated by 2D difference gel electrophoresis (2D DIGE) and analyzed using DeCyder 2D 7.0 Differential Analysis Software. We have found significative changes between the different groups in five spots (p1-p5). Spots p4 and p5 were identified by mass-mass spectrometry as chain A of C3 complement with a C.I. of 100%. These results suggest that C3 Complement may acts as a biomarker of diabetes mellitus type 2 in male patients.

P09-18
Effect of maslinic acid food ration on the hepatic proteome of *Sparus aurata*

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Maslinic acid (MA) is a natural pentacyclic triterpene used as a feed additive to stimulate growth, protein turnover rates, and hyperplasia in fish. To further our understanding of the effects of ration restriction on the cellular mechanisms underlying the action of MA we have used a proteomic procedure based on 2-DE coupled with MS to identify proteins differentially expressed in the livers of juvenile gilthead sea bream (*Sparus aurata*). Four groups of fish of 12 g of a mean body mass were grown under fish-farm conditions and fed 210 days with a 100 mg/kg MA-enriched (MA_{100}) or control diet (control). Two groups were fed *ad libitum* (AL-MA, AL-control) and other two groups were fed at 1.5% total biomass restricted ration (R-MA, R-control). After the comparison of the protein profiles, 49 protein spots were found to be altered in abundance (≥3-fold) in R-MA group versus R-control, and only 10 protein spots were found altered in AL-MA group versus AL-control. Analysis by MALDI-TOF/TOF allowed the unambiguous identification of 29 spots in R-MA, corresponding to 19 different proteins. These proteins were: phosphoglucomutase, phosphoglucose isomerase, *S*-adenosylmethionine-dependent methyltransferase class I, aldehyde dehydrogenase, catalase, 6-phosphogluconate dehydrogenase, fumarylacetoacetate hydrolase, 4-hydroxyphenylpyruvic dioxygenase, methylmalonate-semialdehyde dehydrogenase, lysozyme, urate oxidase, elongation factor 2, 60 kDa heat-shock protein, 58 kDa glucose-regulated protein, cytokeratine E7, type-II keratin, intermediate filament proteins17-β-hydroxysteroid dehydrogenase type 4, and kinase suppressor of Ras1. In AL-MA, the 10 protein spots corresponding to two different proteins were identified as chain A of lysozyme and catalase. Thus, we propose that the synergy between MA feeding and the ration restriction, cause the greatest changes in liver protein expression in gilthead sea bream. This work was supported by research group BIO-157, Junta de Andalucía, and collaborative program between the OTRI of the University of Granada and companies 'Biomaslinic' SL, Granada and the 'Azucarera del Guadalfeo', Division of Marine Aquaculture, Salobreña, Granada.

P09-19
Proteomic profile of thyroid FNA *in vivo* and in post surgery lesions

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Starting from our previous work on fine needle aspiration (FNA) of nodule after surgical thyroid resection, we collected a large number of pre-surgical thyroid FNA samples in order to verify results previously obtained with a proteomic approach. FNA is a technique largely applied in the diagnosis of thyroid tumours but sometimes cytological analysis can't classified as malignant or benign some nodules that have to be subjected anyway to surgical removal.

To confirm the potential biomarkers previously identified in FNA obtained after surgery, we collected 414 pre-surgical samples. Immediately after the FNA procedure on thyroid nodules, the syringe was washed with saline solution and the liquid centrifuged. For each FNA sample a cytological analysis was performed and a histological analysis was also carried out for the patients that underwent surgical intervention.

Samples were pooled according to cytological and histological examination, and submitted to two-dimensional electrophoresis (2DE). The gels were stained with Sypro and images analyzed performing a comparison between carcinoma and control classes. Proteins spots of interest were identified by NanoLC-ESI-MS/MS analysis.

We confirmed the different expression of proteins found in our previous study: L-lactate dehydrogenase B chain, annexin A1, DJ-1 protein, haptoglobin, peroxiredoxin 1 and moesin. Furthermore, we found also new protein spots differentially expressed that are in course of identification.

To have found in the FNA in vivo and in a larger number of patients, proteins previously identified in the after surgical FNA, confirmed the diagnostic relevance of these putative biomarkers. Moreover, we highlighted new proteins that can add information regarding the pathophysiological mechanisms of thyroid tumours supporting the applicability of thyroid FNA as a useful tool in the diagnostic classification of the disease.

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[Correction after online publication 30 August 2012: Abstract text should not be the same as P09-30.]

P09-20
Genome-wide transcriptional response to pesticide exposure in the model yeast *Saccharomyces cerevisiae*

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Diverse frequently used pesticides may have hazardous effects towards ecosystems and human health. To contribute to the development of non-animal alternative bioassays for toxicological assessment of pesticides and, on the other hand, to predict novel mechanistic clues on their effects that may be relevant for microbial and higher eukaryotes from ecosystems, a genome-wide study was carried out using the simple eukaryotic model *S. cerevisiae*. Transcriptional profiles in response to equitoxic concentrations (20% growth inhibition) of four herbicides (alachlor, S-metolachlor, MCPA-ME, diuron), one insecticide (carbofuran) and one fungicide (pyrimethanil) were obtained using Affymetrix GeneChip Yeast Genome 2.0 arrays. Comparison of the datasets of differentially expressed genes for the six pesticides suggested that yeast respond differently to the structurally different pesticides. Analysis is under way to exploit these differences aiming to predict novel mechanisms of yeast response to these pesticides. Interestingly, a number of proteins (~16%) encoded by up- or down-regulated genes shared significant homology with proteins from higher eukaryotes representative of ecosystems inhabitants that may be exposed to pesticides in the field (1). Furthermore, several molecular biomarkers of pesticide exposure and toxicity were identified that may be useful to the development of yeast-based bioassays. In general, transcript levels of selected candidate biomarkers, measured by quantitative RT-PCR, varied consistently with pesticide effects at higher level of biological organization (namely, yeast population growth) (1), which is an essential trait of toxicity biomarkers.

Keywords: environment, pesticide toxicity, stress-response, transcriptomics, biomarkers.

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P09-21
β-oxidation inhibition in *Acinetobacter radioresistens* S13: a strategy to improve carboxylic acids ω-oxidation

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Biodegradable plastics can find applications in several commercial products, so-called 'environmental friendly'. One possibility to obtain these materials is constituted by fatty acids polycondensation; the limiting step for this process is the absence of a second carboxyl or hydroxyl function in a terminal ω- or subterminal ω-1 position of the fatty acids. The most interesting approach to bypass this problem is represented by the use of microorganisms able to grow on fatty acids degrading them by the ω-oxidation pathway and not only by the classical β-oxidation pathway. Preliminary study has revealed that the strain Acinetobacter radioresistens S13 was a potential ω-oxidant bacterium. In this work this ω-oxidant potential was evaluated setting up conditions in which the strain was grown in presence of sodium acetate, pelargonic acid and acrylic acid, a compound described in the literature as a β-oxidation inhibitor. Metabolic analyses by HPLC highlighted the ability of the strain to consume the whole supplied pelargonic acid also in presence of acrylic acid. To evaluate the effective ω-oxidant potential of *A. radioresistens* S13 a comparative proteomic approach was used: two-dimensional electrophoresis maps of the proteins recovered after growth of the strain in three different culture media (sodium acetate as sole carbon source, sodium acetate + pelargonic acid as carbon sources and sodium acetate + pelargonic acid + acrylic acid) were performed. The differentially expressed spots were analyzed by MALDI TOF TOF mass spectrometry. The protein patterns among the three different conditions are very different highlighting that great physiological differences were induced in the strain by the presence of the tested different substrates. As regards the ω-oxidant potential, in the presence of acrylic acid the up-regulation of an aldehyde dehydrogenase and of an alcohol dehydrogenase was observed: it is hypothesizable that these two enzymes may be effectively involved in the ω-oxidation pathway of degradation of pelargonic acid.

P09-22
High-throughput proteomic analysis of mitotic cells and drug-induced mitotic blocked cells

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Antimitotic drugs, such as Microtubule Interfering Agents (MIAs), are one of the most useful groups of compounds currently in use in chemotherapy. Drugs as paclitaxel (Ptx) or vinblastine (Vb) induce cell death after mitotic blockage because of the inhibition of the microtubule dynamics during mitotis, although there are aspects of the molecular mechanisms which remain unknown.

Currently, mitotic blockage induced by nocodazol or some other antimitotic drugs is used as a model to analyze the events that take place during the M phase. Previously results from our lab, using 2DE, showed that some proteins (eEF1Bγ and p54nrb) that are phosphorylated after mitotic blockage are also phosphorylated during normal mitosis. We have also observed that other proteins such as Annexin A2, are specifically phosphorylated in drug-induced mitotic blocked cells.

For this reason and also with the aim of contributing to clarify the mechanisms that are implicated in the mitotic blockage process as well as to search for new antimitotic stress markers, we have compared the proteome and the phosphoproteome of normal mitotic and mitotic blocked cells using high-throughput quantitative technics. For that purpose we have isolated cells in both conditions by Mitotic Shake-Off (MSOff). Lys-C digestion of the proteins and reductive dimethylation (ReDi) labeling of the peptides followed by SCX fractionation and LC-MS/MS analysis using LTQ-Orbitrap Velos has allowed us to quantify 4789 proteins and 8007 phosphosites (after phosphopeptide enrichment with TiO2). Phosphopeptide ratios calibrated by protein levels, allowed us to distinguish real differential phosphorylation from altered protein expression, showing that 15% of the normalized phosphosites ratios are upregulated (about 950 phosphosites) in mitotic blocked cells, including Anxa2, eEF1Bδ, TP53BP1 and

HSP90. In summary, if proteins considered to be changing during mitosis are truly not modified in a normal mitotic process the current strategies used to study and characterize protein changes during mitosis should be revised.

P09-23
Transcription response of European lobster larvae (*Homarus gammarus*) to ocean acidification: The 'evil twin' of global warming

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The oceans have served as a natural sink for up a third of the anthropic-produced CO_2 over the past 200 years. The addition of CO_2 in seawater is driving the carbonate system to lower pH, a process commonly known as ocean acidification. Ocean acidification has become an environmental condition that threatens marine ecosystems. To understand the mechanisms of adaptation that crustaceans face with ocean acidification, we carried out a heterologous, microarray-based transcriptomic analysis of the physiological response of European lobster larvae (Homarus gammarus) to different seawater CO_2 scenarios. Larvae were raised in seawater with 280 ppm CO_2 (similar to pre-industrial conditions) and at a projected condition of 1200 ppm (what is expected at the end of the 21st century). Targeting expression of ~1300 genes involved in several biological pathways, mainly in lysosomes, the phosphatidylinositol signaling system, oxidative phosphorylation, and splicesome. In response to elevated CO_2, lobster larvae changed gene expression, similar to sea urchin larvae energy metabolism, through the electron transport chain. However, protein control synthesis in the lobster larva is impacted greatly at transcription instead of translational control, as echinoderms seem to react.

P09r-24
New roles for Tubulin cofactor E (TBCE) in cell division

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Understanding how polypeptides acquire a functional native configuration is crucial to comprehend the vast majority of genetic diseases. Tubulin heterodimer formation is probably one of the most complex models of protein folding. So far, six proteins have been implicated in the post-chaperonin tubulin folding pathway. These proteins, named the tubulin cofactors (TBCs) TBCA, TBCB, TBCC, TBCD, TBCE and Arl2, have recently been implicated also in the regulation of microtubule dynamics as well as in centrosomal function, centriologenesis and the regulation of primary cilium assembly and disassembly.

In vivo studies demonstrate how both TBCs gene overexpression or silencing results in non viable cellular phenotypes. In most cases, severe microtubule defects including microtubule depolymerization, abnormal spindles assembly or primary cilium and centrosomal aberrations are observed. This is probably why there are very few TBCs gene mutations reported compatible with life.

The Hypoparathyroidism-retardation-dysmorphism syndrome (HRD) and the Kenny-Caffey syndrome (KC) are two rare and fatal human diseases caused by defects in TBCE. A single aminoacid deletion in TBCE has also been reported to produce a progressive motor neuronopathy in mice. This cofactor has been shown necessary for life in all organisms studied and produces massive microtubule depolymerization when overexpressed. Here we investigate the TBCE half-life *in vivo* and present preliminary data of possible roles in cell biology other than participating in the tubulin assembly route. We show the subcellular localization of this protein, and also investigate TBCE gene silencing phenotypes.

P09-25
A tool for biomarker discovery in the urinary proteome: a manually-curated human and animal urine protein biomarker database

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Urine is an important source of biomarkers. A single proteomics assay can identify hundreds of differentially expressed proteins between disease and control samples; however, the ability to select biomarker candidates with the most promise for further validation study remains difficult. A bioinformatics tool that allows accurate and convenient comparison of all of the existing related studies can markedly aid the development of this area. In this study, we constructed the Urinary Protein Biomarker (UPB) database to collect existing studies of urinary protein biomarkers from published literature. To ensure the quality of data collection, all literature was manually curated. The website (http://122.70.220.102/biomarker) allows users to browse the database by disease categories and search by protein IDs in bulk. Researchers can easily determine whether a biomarker candidate has already been identified by another group for the same disease or for other diseases, allowing for the confidence and disease specificity of their biomarker candidate to be evaluated. Additionally, the pathophysiologic processes of the diseases can be studied using our database with the hypothesis that diseases that share biomarkers may have the same pathological processes. Because of the natural relationship between urinary proteins and the urinary system, this database may be especially suitable for studying the pathogenesis of urological diseases. Currently the database contains 707 and 302 records compiled from 237 and 43 publications of human and animal studies, respectively. We found that biomarkers identified by different proteomic methods had a poor overlap with each other. The differences between sample preparation and separation methods, mass spectrometers, and data analysis algorithms may be influencing factors. Biomarkers identified from animal models also overlapped poorly with those from human samples, but the overlap rate was not lower than that of human proteomics studies. Therefore, it is not clear how well the animal models mimic human diseases.

P09-26
Aeromonas molluscorum Av27 transcriptome analysis: future applications for TBT detection and remediation

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Regular surveys have been performed in Ria de Aveiro (NW Portugal) since 1997 that clearly demonstrate that TBT pollution is declining after the implementation of the Regulation (EC) No.

782/2003. However, this pollution is still of great concern at port and shipyard areas where TBT is retained within muddy sediments for many years posing high local ecological risk. Aeromonas molluscorum Av27 is a TBT resistant bacterial strain isolated from Ria de Aveiro. We show that besides its tolerance to high TBT concentrations (up to 3 mM) it is also able to degrade this compound into the less toxic DBT and MBT, and to use TBT as carbon source. Given the properties exhibited by this bacterium, at least two applications can be envisaged: development of a biosensor and remediation of TBT contaminated areas. To fully elucidate the mechanisms underlying the TBT resistance, and since the complete genome of *A. molluscorum* is not available yet, massive parallel pyrosequencing-based approach applied to the transcriptome analysis was employed to reveal all the genes being expressed in the presence of increasing TBT concentrations (50, 500 and 1500 μM TBT). Given the genome size (approx. 4700 kb), three experimental and one control conditions were studied and pyrosequencing, assemblage and annotation were performed. Here we show a number of genes involved in different molecular functions and biological processes that were found to be expressed in the different experimental conditions studied and how this can be explored to the development of a biosensor and for the remediation of TBT contaminated areas. This analysis will be complemented with the study of the expression of specific gene(s), involved in TBT resistance/degradation, by Real Time-PCR.

P09-27
Response of *Escherichia coli* to Berberine

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The irresponsive use of currently available antimicrobials causes reduction or even elimination of their activities. This constitutes a significant menace to public health. Eventually scientists started a search for new antimicrobial classes. Due to their medicinal properties, easy attainability and limitless ability to synthesize substances that serve in plant defense mechanisms against microorganisms, plants have gained importance to fight with the drug-resistant microorganisms. In this study, the aim was to investigate the antimicrobial effect of berberine, a plant alkaloid, on *E. coli* K12 cells. Molecular response of the cells to the inhibitory effect of berberine was screened at transcriptomic level. Cells were harvested at early exponential phase and the major adaptive changes between the control and the drug treated cells were compared. Gene expression profiles of cells were determined using microarray technology. Microarray results showed that a significant number of genes related to catabolic processes were up-regulated. Despite the significant increase in genes related aminoacid biosynthesis, there was a significant decrease in genes related to biosynthesis mechanisms. Addition to that, there was significant increase in the number of down- regulated genes related to membrane proteins and transporter proteins. These results are important in understanding the molecular mechanism of the action of the antimicrobial agent berberine to identify targets for future drug design.

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P09-28
Vitellolytic enzymes of *Culex quinquefasciatus*

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Despite *Culex quinquefasciatus* being an efficient vector of diseases such as lymphatic filariasis, West Nile fever and diverse viral encephalitis, very little research on its embryonic development has been conducted. Like all oviparous animals, the embryonic development of mosquitoes depends on the yolk degradation by vitellogenic hydrolases. Cathepsin-like proteases involved in yolk protein hydrolysis have been identified and characterized in several model organisms.

Here we describe a cathepsin-like activity from egg extracts of *Cx. quinquefasciatus* using benzyloxycarbonylarginyl-arginine 4-methylcoumarin-7-ylamide (Z-Arg-Arg-NHMec) as substrate The optimal temperature and pH for the hydrolysis of Z-Arg-Arg-NHMec is 27 oC and 5.0, respectively. The enzymatic activity is intact after an 18 h incubation of the egg extract at the optimal temperature and pH. SDS-PAGE analysis after this incubation revealed the disappearance of most of the extract polypeptide bands least one of 30 kDa. The addition of E-64 (trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane) to the extract almost entirely inhibited the proteolysis, suggesting that a cysteine protease activity is responsible for the egg proteins digestion under the conditions of our incubation assay.

Mass spectrometry analysis of the remaining 30 kDa band with Sequest® algorithm using a NCBI non-redundant database identified two cathepsins B of *Cx. quinquefasciatus* and, based on them, specific primers were designed. RT-PCR showed that both enzymes have their higher expression between 24 and 48 hours after blood meal uptake. Our preliminary data strongly suggest that *Cx. quinquefasciatus* has two isoforms of cathepsin B which play a key role in the yolk protein degradation.

P09-29
Acute phase characterization by 2-DE/MALDI-TOF MS of bovine serum and whey from healthy and mastitis affected animals

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Acute phase proteins (APP) have been identified in whey and sera from healthy and mastitic cows through the proteomic analysis using two-dimensional electrophoresis (2-DE) coupled with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Although normal and mastitis serum samples show relatively similar protein composition, marked differences in expression levels and patterns can be observed. Conversely, normal and mastitis whey showed a very different composition, likely due to extravasation of blood proteins to the mammary gland. Different isoforms from the most abundant protein in milk, casein, were detected in both normal and mastitis whey. Other proteins, such as lactotransferrin, were only detected in the inflamed animal samples. Immunoglobulins showed different patterns but not increased levels in the inflamed whey. Also, many cellular proteins present in mastitis cow's whey were absent from healthy cow's milk. They are responsible for the great change in composition between normal and mastitis whey, especially those which exert a biological function related to immune defense. Data collected in this work are of interest for gaining information about physiological changes in protein patterns in different fluids as a result of an acute phase process in farm.

P09-30
An oligonucleotide-based microarray to detect and monitor mobile genetic elements

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Insertion sequences (IS) are regarded as the simplest type of transposons and, frequently, they contain a unique gene coding for the transposase required for their proliferation (by transposition) within the host genome. IS are modular elements that, when expanded, can promote recombination and chromosomal rearrangements, contributing to genome plasticity and genetic variability in bacterial populations.

We have designed and constructed an oligonucleotide-based microarray of transposase genes present in the genomes of acidiphilic organisms. This microarray has allowed us to detect and quantify changes over time in the population of transposases of bacteria in environmental samples from the Tinto river (Huelva), an extremely acidic (pH 2) habitat. We have also used the array to detect expression of transposase genes.

Additionally, we have used the microarray to monitor changes in genomic IS abundance in a strain of Acidiphilium sp. grown in culture over three years (2007–2011). Acidiphilium sp. contains a large number of transposases in its genome which probably contribute to genomic plasticity. Although most ISs remained stable over this time period, we observed the disappearance of some ISs and the proliferation of others.

The results indicate that the IS microarray is a useful tool for studying the dynamics of transposons and other mobile elements in bacterial populations.

P09-31
Single nucleotid polymorphisms in relation to reduced bone mineral density and osteoporotic fractures in a young Caucasian woman

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Osteoporosis is a common disease which is characterised by reduced bone mass and increased risk of fracture. Osteoporosis is a highly heritable trait. Many candidate genes have been proposed as being involved in regulating bone mineral density (BMD).Candidate genes which have been studied in relation to BMD and osteoporotic fractures include collagen type1 gene, calcitonin receptor gene, estrogen receptor gene and vitamin D receptor gene .We present here a case of 32 years old a young woman who is Caucasian origin, who lives in Diyarbakır now. Her BMD in the lumbar spine and femur were −3,2 T score and −2,4 T score respectively. We used Dual x-ray absorptiometry(DEXA) for measuring BMD . We used (Clinical arrays MetaBone assay) to detect polymorphisms. This method is based a low density chip at the bottom of an classical 2 ml tube. DNA was extracted from blood with EDTA. DNA amplification, denaturation, hybridization and the other steps were carried out respectively. Biochemical analysis, complete blood analysis and complete hormonal measurements were recorded to exclude an underlying secondary cause of osteoporosis. We analysed Col1A1-SP1, polymorphism for collagen type1 gene, CTR-ALU1 polymorphism, for calcitonin receptor gene, ESR1X-XBAI, ESR1P-PVUII polymorphisms for estrogen receptor gene, VDRF-FOKI, and VDRB-BSMI polymorphisms for vitamin D receptor gene. The analysis showed us that her genotype was *SS, aa,PP, Xx, BB, FF*. (Normal genes are indicated by capital letters). Positive associations between these single nücleotide polymorphisms (SNP's) and bone density were reported by several studies. These type of studies will help us to take clinical desicion and support certain therapies, especially for early age fractures and early treatment for bone prevention.

Keywords: osteoporosis, BMD, estrogene, vit D, calcitonin, receptor gene, Col1A1, SNP.

P09-32
Saccharomyces cerevisiae genome-wide screen for the identification of K2 killer toxin effectors

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Biotoxins are widespread molecules involved in microbial pathogenicity and self-defense mechanisms, directly relevant to human health and the food industry. Understanding how they work is of prime importance to clinical and applied research. The budding yeast (*S. cerevisiae*) killers serve as a good model to study the activity of biotoxins. The characterization of such toxins has consistently provided significant insights into the basic mechanisms of self-defense and therefore immunity, in the mechanisms of virus-host cell interactions and toxin entry into eukaryotic target cells.

In order to identify gene products modulating the sensitivity to K2 killer toxin, we conducted several concurrent genome-wide screens in *S. cerevisiae* and identified 205 genes involved in resistance and 127 in sensitivity. We conclude that the gene products involved in resistance and sensitivity strikingly belong to distinct gene ontology families. Importantly, most genes identified in our screens (∼70%) had not previously been linked to the biology of K1 and K28 killer toxins, indicating strong specificity towards K2 toxin. K2 effectors are involved in cell wall structure/biogenesis, stress-induced cell signaling, ion and pH homeostasis, and the generation of ATP. Our work demonstrates that despite the fact that K1 and K2 toxins share some aspects of their killing strategies they rely on strikingly different molecular actors to do so.

P09-33
Aberrant Ring finger protein 43 (RNF43) and their proliferative role in cholangiocarcinoma

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Cholangiocarcinoma (CCA) is devastating cancers and is increasing both worldwide incidence and mortality rate. A mutation

landscape of CCA had been demonstrated that beside the major cancer genes including TP53, KRAS and SMAD4, there is a novel mutated gene, RNF43, which was shown poor prognosis in patient bearing RNF43 mutation. Single nucleotide polymorphism (SNP) may play a more modest role in CCA susceptibility. We determined genetic variation in RNF43 of 52 CCA patients by PCR based – Capillary Sanger sequencing for all their coding sequence. High frequency of RNF43 polymorphism were found on exon 2 (Rs3744093 C > T; 36/52) and exon 3 (Rs2257205 A > G; 40/52) respectively. To determine whether RNF43, RING-type E3 ubiquitin ligase, involve in cell growth and survival, RNAi of the gene was performed. Knocking down of RNF43 in CCA cell lines (M214 and M156) lead to cell cycle arrest in G1. We also found that silencing RNF43 encouraged increasing level of p53 dependent transcription of p21. These preliminary data further elucidate the potential risk of genetic variation in RNF43 for CCA susceptibility and suggest that p53 pathway may regulated by RING – type E3 ubiquitin ligase in tumor progression and survival.

P09-34
Effect of silver nanoparticles at molecular level in the marine diatom *Thalassiosira pseudonana*

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Silver nanoparticles (AgNP) are among the most frequently used nanomaterials due to their antiseptic properties (1). Their application ranges from the industrial to the medical field and the release of silver nanoparticles to the aquatic environment is a major concern (2). It is expected that the use of nanoparticles will further increase in the future. Consequently, it is crucial to assess the potential toxic effects of AgNP on the aquatic organisms. In our studies the diatom Thalassiosira pseudonana, an ecological relevant organism (3) was used to investigate the effect of silver nanoparticles. We showed that the AgNP inhibited the growth of the diatoms and similar studies were performed as well with silver nitrate ($AgNO_3$). To investigate the mechanism of toxicity, we studied the response at molecular level. Based on the observed growth inhibition, concentrations of 10 and 1 μM for AgNP and 1 and 0.1 μM for $AgNO_3$ were selected for DNA microarray, corresponding approximately to the EC50 and 1/10 EC50, respectively. The transcriptomics profiles of AgNP and silver nitrate were compared and linked to the observed cytotoxicity, to understand the metabolic pathways involved and potentially to identify molecular biomarkers.

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P09-35
Proteomic profiles of gastro-intestinal neuroendocrine tumors

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Introduction: Neuroendocrine tumors are rare tumors of the interface between the endocrine and nervous system. Digestive tract neuroendocrine tumors account for 95% of all neuroendocrine tumors. In gastro-intestinal tract, carcinoids represent 20% of all digestive neoplasms and 6% of colorectal neoplasms. The estimated incidence is 8–100 000 individuals. Materials and Methods: In our study we investigated 22 cases of neuroendocrine digestive tumors hospitalized in Emergency Clinical County Hospital of Constanta. In these patients we determined pathologic and proteomic profiles of neuroendocrine tumors using markers like synaptophysin, neuron-specific enolase, Ki-67, PGP 9_5, chromogranin and broad- spectrum cytokeratins. Results: Our data suggested that neuroendocrine digestive tumors present variable localization with large intestine predominance. Grading of neuroendocrine tumors correlate with loco-regional and vascular invasion and positive diagnosis of neuroendocrine tumors was emphasized by immunohistochemical profile of these tumors. Primary tumor was identified in 95% cases and in 5% of cases was identified carcinoid invasion. Immunohistochemical profile for synaptophisine was intense positive in 58% of cases and moderate positive in 42% of cases regardless localization. Chromogranin A expression was moderate positive in 42% of cases, low positive in 40% of cases and negative in 18% of cases (neuroendocrine tumors with large intestinal localization). Conclusions: Neuroendocrine digestive tumors present variable localization with large intestine predominance localization. Grading of neuroendocrine tumors correlate with loco-regional and vascular invasion. Positive diagnosis of neuroendocrine tumors was emphasized by immunohistochemical profile of these tumors.

P09r-36
Effect of endoglin isoforms on the monocyte proteome composition. Functional implications

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Endoglin (CD 105, TGF-β receptor III) is a homodimeric transmembrane glycoprotein that plays a crucial role in vascular remodeling and angiogenesis and is involved in important physiopathological processes such as hereditary hemorrhagic telangiectasia (HHT), preeclampsia or cancer. Two different alternatively spliced isoforms of endoglin have been reported, L-endoglin and S-endoglin. Endoglin expression is up-regulated during the monocyte/macrophage transition, but little is known about its role in the immune system. Interestingly, an increased expression of the S-endoglin isoform during senescence of the monocyte-macrophage lineage, in both human and murine models, was observed. To assess the individual effect of endoglin isoforms on the monocytic lineage, we performed a stable isotope labeling of amino

acids in cell culture (SILAC) analysis of both L-endoglin and S-endoglin transfectants in the human promonocytic cell line U937. By differentiating the monocytic endoglin transfectants into macrophages, we have also analyzed the secretome, focusing on the cytokine expression patterns in order to study macrophage polarization and the role of endoglin in the immune system during aging. Our functional validation studies suggest a non-redundant role for each endoglin isoform on the monocyte biology.

P09-37
Characterization of the Staphylococcal autolysin Sal in Staphylococcus aureus

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Staphylococcus aureus is a Gram+ human pathogen responsible for a wide range of infections, from minor skin infections to life-threatening diseases such as pneumonia, toxic shock syndrome and sepsis. Itis emerging as an important medical need, since infections by staphylococcal strains resistant to antibiotics often result in diseases difficult to be treated and resolved. The *Staphylococcal autolysin* (Sal) is one of the most studied surface proteins in *S. aureus*. It is a peptidoglycan hydrolases, exported to the bacterial surface as an inactive precursor and then, through a proteolytic cleveage, it is processed in two active extracellular enzymes: an *N*-acetylmuramoyl-L-alanine amidase (62 kDa) and an endo-β-*N*-acetylglucosaminidase (52 kDa) corresponding to the N-terminal and the C-terminal region of the precursor, respectively (1). This enzyme plays several roles in staphylococcal biology such as autolysis in response to antibiotic treatments, cell wall remodeling and cell wall division after duplication and, although a lot of information is available on Sal functions, very little is known about the molecular mechanisms involved in its activation and in its processing. In the present work, we describe the expression profiles of Sal precursor and its mature enzymes during bacterial growth and after exposure to stress conditions (e.g. in the presence of antibiotics) using western blot analysis followed by mass spectrometry. Experiments using a TAP-MS approach are ongoing in order to investigate staphylococcal proteins possibly involved in Sal maturation and processing.

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P09-38
Global proteomic analysis of outer membrane vesicles derived from the probiotic *Escherichia coli* strain Nissle 1917

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Escherichia coli Nissle 1917 (EcN) is a harmless probiotic, gut-colonising strain of faecal origin, which displays beneficial effects on several types of intestinal disorders. This strain is able to establish itself in the human intestine and out-compete pathogens by expressing numerous fitness factors such as iron-uptake systems, adhesins, proteases and microcins. Most of these factors are encoded by strain-specific genes mainly clustered in four large islands. As other Gram-negative bacteria, EcN produces outer membrane vesicles (OMVs) that can disseminate far from the cell and play important roles in growth, survival and interaction with the environment. In this study we have performed proteomic analysis of OMVs from EcN cultures grown in LB broth. OMVs were obtained by ultracentrifugation from the cell-free supernatant and subjected to SDS/PAGE. After Sypro Ruby® staining, protein bands were excised and analysed using the liquid chromatograph nanoAcquity (Waters) coupled to a mass spectrometer Orbitrap-Velos (Thermo Scientific). Three independent analyses allowed identification of more than 70 proteins. Most of them are involved in cellular processes, metabolism, and information storage and processing, and had been previously described to be present in OMVs from *E. coli* laboratory strains. We also identified some specific fitness factors involved in iron-uptake systems like the ferric aerobactin receptor IutA or adherence and invasion-related proteins such as S-fimbrial subunits SfaH or SfaG. Remarkably, OMVs also contain proteases like MltA which might be involved in antimicrobial activity. The identification and further characterization of these particular proteins are important to better understand colonisation mechanisms and probiotic features of EcN.

P09-39
Proteomic evaluation of preservation solutions prior to kidney transplantation

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Organ failure is mostly resulted in organ transplantation such as liver, kidney, heart and this still continues to be a major risk for public health. Kidney transplantation is by far the most frequently carried out transplantation globally. In clinical practice, kidney is kept in preservation solution until it is transplanted to the recipient. Here, we focused on the fact that some bio-molecules and/or peptides, enzymes are released to preservation solution from the organ during cold ischemia period. In this study, it was aimed to investigate the bio-molecules with biomarker potentials released from kidney to preservation solution. Preservation solutions from twenty donors were collected before kidney transplantation. Using the human plasma as control group, the proteins of interest were determined by two-dimensional gel electrophoresis (2-DE) and subsequently analyzed by MALDI-TOF-MS. Nano-LC-MS/MS was used to analyze the preservation solutions as a second approach. The solutions were subjected to trypsin digestion and obtained peptide mixture was separated by the 1.7 μm BEH C18 0.75 μm × 250 mm nano-LC column followed by the analysis with Waters SYNAPT-HDMS system. The results of LC-MS/MS method were correlated with 2-DE method. Using LC-MS/MS enabled us to identify proteins with very low concentrations. On the other hand, 2-DE method resulted on identification of about 150 proteins whereas only 30 proteins were identified using LC-MS/MS method. Combining two proteomic approaches, proteins released from organs to preservation solution during cold ischemia period were identified. Each identified protein is a putative biomarker candidate to predict the viability of the organs before transplantation. This study was financed by The Scientific and Technological Research Council of Turkey (TUBITAK), project number 110S103.

P09-40
Pfam-based survey of prokaryotic transposases in completely sequenced genomes

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Transposase genes have been identified as the most abundant genes in nature. They code for enzymes that are responsible for the proliferation of Insertion Sequences (ISs) within genomes. The presence of multiple copies of a given IS can promote the occurrence of genomic rearrangements. ISs can spread across genomes by horizontal gene transfer and can mediate the mobilization of selectable determinants, such as antibiotic resistance or virulence genes. Therefore, IS activity plays an essential role in evolutionary processes at all time scales, from short term adaptation to long term speciation. ISs have often a simple structure that consists in a single transposase gene flanked by inverted repeat (IR) sequences. According to ISfinder, the reference database for archaeal and bacterial ISs, they have been classified into about 25 different families on the basis of transposase similarity and overall organization (Siguier *et al.*, 2006, NAR 34:D32). In this work we report the use of HMM profile-based searches to perform a survey of prokaryotic transposases in completely sequenced genomes. A collection of 4 425 920 proteins, derived from 1499 genome sequencing projects, was re-annotated by comparison against 13 672 profiles, representing the complete Pfam 26.0 collection of protein domains. Predicted protein domains were assembled to reconstruct protein architectures that were then screened against a reference collection of 283 transposase architectures. The reference collection was constructed on the basis of (i) Pfam domain annotations and (ii) domain composition and architecture of the transposases included in the ISfinder database. A total of 59 391 transposase genes were identified and a database was generated. The distribution of transposase genes and architectures was studied at various levels, from individual genomes to general taxonomic groups.

P09-41
Protein-protein interaction analysis of Ter proteins

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The analysis of the protein-protein interactions is one of the possible approaches how to obtain important information about localization and function of the corresponding protein partners inside the cell. Resistance to tellurium is a common feature for many microbial pathogens. Until now, five genetic determinants of tellurite resistance have been characterized but the molecular principle mediated the resistance still remains an obscure. The four genes essential for preserving the resistance – *terB*, *terC*, *terD* and *terE* has been previously cloned into pLK18 plasmid that was proved to confer the resistance for pottasium tellurite in *E. coli* host strain. However, very little is known about biochemical activities and properties of the gene products thus far. It has long been suggested these four Ter proteins form a membrane-localized complex. By using Duet Vector co-expression system, cell fractionation and immunochemical detection we showed co-localization of all Ter proteins in membrane fraction in contrast to the control samples with only one protein produced. We subsequently employed BACTH two hybrid system to confirm interactions between all four Ter proteins *in vivo* using various combinations of the partners and original pLK18 plasmid. These interactions were further characterized by PPI bioinformatics analysis and two hybrid system with partial fragmentation of the corresponding *ter* genes. The results of our work thus support the formation of multiprotein Ter complex and propose the most accurate models of interaction.

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P09-42
Spermiomics: a combined tools array designed for the global survey of sperm function

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The study of sperm biology is a very complex field with many unanswered questions at this moment. The main cause for this is the great complexity of the sperm life, which is translated in the existence of many, concomitant and complex molecular mechanisms that modulate sperm function during all of their lifespan. The overall study of this complexity is not completely possible with classical analytical tools, which are based on the analysis of concrete, punctual functional aspects. In this way, only the application of integrated analytical systems, integrating analysis systems like metabolomics and miniarray studies, will render a better and deeper knowledge of the mature mammalian sperm functionality. In this way, the coordinated utilization of these integrated analytical systems was named 'spermiomics'. In this manner, spermiomics would be the best tool for future investigations of the overall, mature mammalian sperm function.

P09-43
Proteomics analysis of Ter protein complexes

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Multi-protein complexes play key roles in many biological processes, as well as in tellurite resistance. Previous analyses showed proteins TerB, TerC, TerD and TerE are essential for conservation of resistance and are suggested to form a stable complex in the inner cell membrane. Here we present the use of chemical cross-linking combined with MALDI-ToF mass spectrometry for characterization of Ter protein complexes. In our work we used Duet expression vectors to produce essential Ter proteins with an N-terminal His tag in a relevant cell type containing a minimal replicon of tellurite resistance. Loss of specific components of Ter protein complexes was prevented by chemically cross-linking by DSP reagent. Basic strategy for increasing specificity and decreasing background was tandem affinity purification of tagged proteins that dramatically improved the signal-to-noise ratio via the generation of clearer sample. The samples complexity was high, therefore gel separation was necessary. In characterizing the binding partners for essential Ter proteins using MS, the major challenge was to identify interacting partners versus sample con-

taminants. The mutual interactions between essential Ter proteins and potential partners will be confirmed by two-hybrid system.

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P09-44

Phage display as a tool for analysis of tellurite resistance determinant from *E. coli* KL53 in the view of the intracellular interactions of the Ter proteins

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This project is focused on the *ter* operon from uropathogenic strain *E. coli* KL53 at the level of proteins. We are looking for their potential cell interacting partners and we hence want to clarify their functions in the process of Te^R and determine the role of *ter* operon in the pathogenicity of microorganisms. At least five Te^R determinants have been identified. However, the molecular principle mediated the resistance still remains unclear. Analysis showed the genes *terB*, *terC*, *terD* and *terE* are essential for conservation of the resistance. All four genes have been subcloned to the plasmid pLK18 and their function has been subsequently verified in *E. coli* host strain. Little is known about the exact mechanism by which these genes mediate the resistance and the identification of protein-protein interactions is a starting point. To achieve this we decided to use phage display. In this work was used commercially prepared kit Ph.D.™-12. We found several peptides representing sequences that may be part of the interactions partners, or their domains for each expressed Ter protein. Our results particularly correlated with the results of previous analyses (Valkovičová, unpublished). Based on these results, several proteins were selected to be tested *in vivo* by two-hybrid system.

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P09-45

Extracellular proteome of *Cellulomonas fimi* grown on different carbon sources

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Lignocellulosic biomass (LCB) is a renewable and abundant resource with great potential for bioconversion to value-added bioproducts. Bacteria able to produce enzymes exploitable for LCB conversion are of extreme importance in the field of renewable energies, providing means to a 'greener' technology. Researchers are now focusing on the use and improvement of these enzymes for use in the biofuel and bioproduct industries. The aerobic soil bacterium *Cellulomonas fimi* produces noncellulosomal carbohydrate-degrading enzymes. In this study we evaluated the expression of cellulosolytic enzymes secreted by *C. fimi* in order to discover some novel bacterial strategies which could advance the field of biorefining and in particular of 2nd generation bioethanol. We performed a proteomic analysis to evaluate the influence of two parameters, time and substrate, on the protein expression by *C. fimi*. We cultured the microorganism for 14 and 25 hours in presence of three different carbon sources: alpha-D-Glucose, Barley Beta-glucan and Sodium Carboxymethylcellulose. Thus we obtained six reference maps of the *C. fimi* secretome: extracellular proteins were isolated from culture supernatants and resolved by two-dimensional electrophoresis. Proteins were identified by LC-MS/MS (ESI-Q-TOF). We also characterized cellulase activity of the secretomes by zymography and DNS assay. We observed that *C. fimi* grown on different substrates and times varied protein production and secretion both in qualitative and quantitative terms. *C. fimi* produces and secretes multiple enzymes to use a wide range of lignocellulosic substrates as carbon sources, showing the enzyme diversity that can be incorporated into its cellulolytic system. This work was supported by Regione Piemonte grant (Misura INT2 n.53/2008).

P09-46

Mucoid morphotype variation of *Burkholderia multivorans*: adaptation to cystic fibrosis lung environment

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Burkholderia cepacia complex (*Bcc*) bacteria are opportunistic pathogens infecting cystic fibrosis (CF) patients. Due to their large genomes, *Bcc* strains are able to survive under the stressful environments and to adapt to ever-changing conditions. Long-term infection of CF patient airways with *Bcc* has been associated with the emergence of phenotype variation (1). Here we studied two clonal *B. multivorans* clinical isolates –mucoid D2095 and nonmucoid D2214– that differ phenotypically due to the production of exopolysaccharide. Expression profiling of mucoid and nonmucoid isolates revealed decreased expression of genes encoding virulence traits and metabolism in nonmucoid isolate, reflecting lower metabolic needs and attenuation of pathways not required for survival (2). As a result, D2214 showed reduced motility but increased biofilm formation, particularly under microaerophilic conditions and a higher long-term survival in minimal medium. These findings suggest that D2214 may reflect an adaptation of *B. multivorans* to the depleted lung environment. Nonmucoid morphotype variation in *B. multivorans* was shown to be triggered by nutrient limitation, antibiotic, osmotic, oxidative and nitrosative stresses. Furthermore, colony morphotype variation within the *Burkholderia* genus occurred in *Bcc* and non-*Bcc* strains irrespectively of clinical or environmental origin. *Galleria mellonella* infection showed that half the variants remained less virulent while the other half showed a mortality rate similar to the parental mucoid isolates. These findings evidence *Burkholderia* ability to adapt and survive in adverse conditions which may have implications in persistence in the human host.

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P09m-47
Comparative proteome analysis of secreted proteins from insulin-resistant C2C12 cells

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Skeletal muscle accounts for majority of insulin-stimulated glucose disposal and has a high capacity to metabolize fatty acids. Impaired glucose metabolism and lipid metabolism in skeletal muscle are hallmark features of insulin resistance associated with obesity, type 2 diabetes, and metabolic syndrome. Elevated plasma free fatty acids (FFA) are thought to be responsible for development of insulin resistance however the mechanisms by which FFA cause insulin resistance are not clear.

Skeletal muscle has been suggested to be source of secreted proteins which can influence metabolism and other biological processes in a systemic manner. Here we report a secretome of an insulin- resistant muscle cell line. To analyze proteins secreted from insulin-resistant murine C2C12 skeletal muscle cells, we applied a stable isotope labeling by amino acids in cell culture (SILAC) based quantitative proteomics platform. Exposure of isotopicaly labeled C2C12 cells to 0.5 mM palmitate results in development of insulin resistance (confirmed by impairment in insulin signaling). Thereafter, we compared the secretome of control versus insulin-resistant cells. Protocols were optimized to efficiently derive secreted proteins from cells in culture. In supernatants, we identified and quantified 2205 proteins including 36 cytokine signaling proteins, 60 growth factors and 46 metallopeptidase. We found that 182 of these proteins were significantly different in palmitate treated cells compare to non-treated cells. In addition to previously reported secreted protein, we identified many novel proteins that have not been shown to be released from skeletal muscle.

These proteins may act as signaling mediators to other cells and tissues and supporting a role of skeletal muscle as an important secretory organ.

P09-48
Transgenic overexpression of the Nrf2 in the spontaneously hypertensive rat ameliorates oxidative stress and features of metabolic syndrome

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Insulin resistance, the central feature of metabolic syndrome, is accompanied by oxidative stress. The spontaneously hypertensive rat (SHR) is the most widely studied animal model of essential hypertension and metabolic syndrome. To test the role of oxidative stress in metabolic disturbances we derived a SHR transgenic line with ubiquitous expression of the mouse *Nrf2* (Nuclear Factor-erythroid 2-related factor2) gene which codes for a key transciption factor with a central role in cellular defence against oxidative stress. Expression of transgene in multiple tissues was associated with significantly increased activities of antioxidant enzymes and reduced levels of TBARS in the liver and kidney compared to SHR nontransgenic controls (0.96 ± 0.10 versus 1.64 ± 0.17 nM/mg and 0.55 ± 0.04 versus 0.69 ± 0.05 nM/mg, $p < 0.05$, respectively). In addition, transgenic rats showed significantly increased levels of α- and γ-tocopherol (52.6 ± 0.5 versus 34.5 ± 3.9 mg/l, $p < 0.001$ and 1.53 ± 0.02 versus 1.23 ± 0.07 mM, $p < 0.01$, respectively). Reduced oxidative stress was associated in transgenic rats with increased sensitivity of adipose and muscle tissue to insulin action when incremental (insulin stimulated minus basal) incorporation of glucose into adipose tissue lipids and muscle tissue glycogen was significantly higher when compared to nontransgenic controls (90 ± 17 versus 43 ± 6 nmol gl/mg prot./2 hour, $p < 0.05$ and 313 ± 48 versus 151 ± 35 nmol gl./g/2 hour, $p < 0.05$, respectively).

These findings provide evidence for an important role of oxidative stress in the pathogenesis of metabolic disturbances in the SHR.

P09-49
Molecular and genetic studies of botrydial biosynthetic pathway: A toxin involved in the infection mechanism of the phytopathogen fungus *Botrytis cinerea*

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Botrytis cinerea is a well-known pathogen affecting a number of commercial crops, which produces a variety of structurally diverse metabolites. There is no evidence for the production of host-specific toxins by this fungus, which is in accordance with the broad host range of this pathogen. *B. cinerea* produces two series of phytotoxic metabolites: a family of sesquiterpenes which contain the basic botriane skeleton and a family of polyketides. Advances in genomic studies, in combination with increased access to DNA sequencing, are providing a wealth of information about how natural products are assembled, mechanisms by which natural product gene clusters can be manipulated to yield new product diversity, and the genetic potential of individual organisms. In the last years we have undertaken the functional characterization of the botrydial gene cluster, which permitted us to complete the biosynthesis of one of the most important toxins excreted by the phytopathogen fungus *B. cinerea* during the infection process. In this communication we report the functional characterisation of the *BcBOT1-5* genes and the encoded key enzymes which catalyze all steps in the biosynthesis pathway leading to botrydial and its derivatives.

P09-50
The role of p53 DNA structure-selective binding in brain cancer

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Brain tumors are highly invasive types of cancer. The inactivation of specific genes together with point mutation in tumor suppressor *TP53* gene potends poor prognosis and dismal survival. Mutant p53 proteins acquire a -gain of function- activity that mainly contributes to tumor aggressiveness by regulation of a specific set of growth and metastasis promoting genes.

In our work we were combined molecular biology and bioinformatics to investigate the role of mutant p53 binding to geno-

mic repetitive regions forming non-B DNA structures in regulation of mutant p53 target genes. Formation of non-B DNA structure is DNA topology dependent. To investigate the importance of DNA topology on mutp53-DNA recognition *in vitro* and *in vivo*, we analyzed the interaction of seven hot-spot mutp53 proteins with topologically different DNA substrates using a variety of electrophoresis and immunoprecipitation based techniques *in vitro*. The binding of mutp53 to repetitive DNA was detected also *in vivo*. Furthermore, the level of mutp53-dependent repression of several mutp53-regulated promoters was influenced by DNA topology. Possible roles of mutp53 binding to non-B DNA in brain tumors are discussed.

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P09r-51
Crz1 and Ca^{2+}-signaling in *Saccharomyces cerevisiae* exposed to arsenic stress

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Arsenic is a highly toxic metalloid widespread in the environment. To cope with arsenic stress, organisms have developed a myriad of protection mechanisms including arsenic detoxification and the maintenance of redox homeostasis disturbed by arsenic compounds. In this work we show that supplementation of media with Ca^{2+} enhances tolerance of wild type and arsenic-sensitive *yap1* mutant strains exposed to arsenic. Crz1 is essential in this process since its disruption impairs the acquisition of tolerance mediated by Ca^{2+} as well as confers a moderate degree of sensitivity to the cells subjected to the metalloid. In addition, our work provides evidences that arsenic elicits a transient increase of free cytosolic Ca^{2+}, which in turn leads to Crz1 nuclear compartmentalization, and culminates with the transcriptional activation of the cell wall biosynthetic gene *GSC2* and genes encoding Ca^{2+} transporters, such as *PMR1* and *PMC1*. Besides, the disruption of the Ca^{2+} import system involving Mid1/Cch1 compromises the acquisition of cellular tolerance to arsenic. Taken together, these data establish, for the first time, that activation of Crz1 and Ca^{2+}-signalling pathways contributes to yeast adaptation under arsenic stress conditions.

P09-52
Studies of the Yap8 – DNA interaction

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The genome of the budding yeast *Saccharomyces cerevisiae* contains the Yeast AP-1 like family, of b-ZIP proteins, Yap1 to Yap8 with similarity at the DNA binding domain and leucine zipper. In all the Yap family members as well as in the fission yeast transcription factor Pap1, the Yap1 orthologue, a conserved sequence of amino acids NXXAQXXFR, in the basic region is found. Pap1 conserved residues interact with the DNA, making direct or water mediated interactions with the base pairs. The conserved Asn86 in Pap1 and Asn235 in GCN4 are important for the interaction with DNA, although they bind with different specificity. Some of these amino acids of Yap8 are replaced e.g. the Asn is substituted by a Leu at position 26. Another striking difference is that Yap1 to Yap3 recognize TTACTAA, designated the Yap responsive element (YRE), whereas Yap8 binds only TGATTAATAATCA a sequence present in Yap8 targets (*ACR2* and *ACR3*). We have used an *in vivo* assay in which the upstream activating sequence of histidine reporter gene was replaced by the sequence under study. The overexpressed Yap, or mutant Yap, either recognized this sequence, activating transcription, or not. Of the constructed Yap mutants, three no longer recognized the DNA, one of which is the mutant L26A. This result was confirmed by electrophoretic mobility shift assay using the recombinant protein with only the predicted basic and leucine zipper regions. The assays described above also showed that a mutant with two of the Yap8 specific amino acid residues replaced with Yap1residues (L26N-N31R) not only recognizes Yap8 13bp sequence but also the YRE. The results obtained so far suggest that the replacement of Asn by Leu26 in Yap8 confers specificity to the DNA recognition by this transcription factor. Further studies, using surface plasmon resonance are being carried out as to allow the determination of the equilibrium dissociation constant (K_D) of the binding of the 13bp DNA to the wild type and mutant proteins.

P09-53
Detection of wilms' tumor 1 (WT1) gene expression in renal cancer cell lines

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The Wilms' tumor 1 (WT1) gene located at chromosome 11p13 normally provides instructions for making a protein that is involved in the development of the urogenital system. Initially this gene was discovered as a tumor suppressor gene. Further studies however show evidences that WT1 is an oncogene. Gene mutation of WT1 was observed in children with renal neoplasm. Studies in certain types of lung, prostate, breast and ovarian cancer show abnormal expression of the WT1 gene. The present study aims to investigate the expression of the WT1 gene from renal cancer cell lines (KDN 1, KDN 2 and KDN 5) and its proliferative activity before and after treatment with suberoylanilide hydroxamic acid (SAHA), a histone deacetylase. Ribonucleic Acid (RNA) from renal cancer cell lines was extracted using Purelink RNA extraction kit and was quantified by Quibit 2.0 Fluorometer. Expression of WT1 mRNA profile will be quantified and analyzed by qRT-PCR. Results obtained from this study is expected to show downregulation of WT1 expression levels that leads to decrease proliferative activity of renal cancer cell lines after treatment with SAHA. This study will give contribution in understanding the role of WT1 gene in the tumorigenesis of renal cancer.

(On-going research, complete results and data analysis will be finished by August 2012).

P09r-54
Evaluation of cloned genes for quantification control in Real-Time PCR assays for infection diseases of veterinarian importance

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The aim of this work was to develop plasmids for using as positive controls in novel own-designed qPCR diagnostic kits. Construction of cloned genes for properly normalization is necessary to obtain reliable expression data related to the presence and quantity of specific pathogen agents of a particular infectious disease. We developed and constructed seven plasmids using gene-specific regions of pathogenic microorganisms: *Mycoplasma agalactiae; Mycoplasma bovis; Mycoplasma ovipneumoniae; Coxiella burnetii; Rabbit enterophatogen Escherichia coli; Listeria monocitogenes; pathogenics Leptospiras*. For each construction, we designed specific primers using NCBI blast tool. All plasmid DNA were synthesized by PCR using a TA type vector. Once we obtained clones, we sequenced them (SecuGen) to verify no mutation through comparing them with original database sequence (CodonCodeAligner software). All Real-Time PCR was performed using an 'Applied Biosystems StepOne' system and incubated in a 48-well low profile plate at 95°C for 10 min, followed by 49 cycles of 95°C for 10s and 60°C for 1 min. The specific amplification curves obtained tackle with all panels which contain reference strains, field strains and clinical samples. Efficiency values obtained for real-time PCR amplification of seven cloned genes ranged from 100.42 to 107.13. Thus, amplification efficiency was near the theoretical optimum level of 2 Efficiency values (E). Slope values and correlation coefficients (R^2) for each assay were from −3.22 to −3.16 and from 0.992 to 0.998, respectively. Cq for seven assay ranged from 8.71 to 37.51. Therefore, all of them got high title values and remarkable quality.

P09-55
Search for coherent gene modules predictors of *Streptococcus pneumoniae* invasiveness

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Streptococcus pneumonia is a pathogenic bacteria responsible for several human diseases, such as pneumonia, meningitis and sepsis. Any pneumococcal disease is preceded by an asymptomatic colonization stage in the human nasopharynx. The transition from colonization to invasion is known to depend on both human and pathogen factors. This work aims to computationally identify pneumococcal genetic factors that influence the likelihood of invasion events. For this purpose, we analyze microarray based comparative genomic hybridization data of 72 pneumococcal strains. Each strain was classified as Invasive, Neutral or Colonizer according to a previous study that compared the frequencies with which strains were recovered from an asymptomatic carrier or from invasive disease episodes. We propose to select genes that, individually or in a coordinated way, affect the frequency of invasion transitions among all colonization events, which we denominate as invasiveness. To detect coordinated sets of genes, we developed a method that uses networks of known interactions between genes to find gene modules that predict invasiveness. Each module is founded with a single gene and then grown with its closest neighbors in the network. The growth is stopped when it no longer adds any predictive power to the module. Each module is then evaluated by statistical significance and robustness to data variability. We tested the method with two different networks. The first network we used was based on a distance score that integrates gene co-occurrence and dissimilarity of association with invasiveness. The second is a network of gene co-expression built from publicly available data sets. Among others functions, the found modules implicate sugar transport and metabolism in pneumococcal invasiveness.

P10 – Glycobiology

P10-1
SweetBac: a new approach for the production of mammalianised glycoproteins in insect cells

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Recombinant production of therapeutically active proteins has become a central focus of contemporary life science research. These proteins are often produced in mammalian cells, in order to obtain products with post-translational modifications similar to their natural counterparts. The baculoviral insect cell system has proven to be a powerful alternative for the expression of a wide range of recombinant proteins in short time frames. The major drawback of baculoviral systems lies in the inability to perform mammalian-like glycosylation required for the production of therapeutic glycoproteins. In this study we integrated sequences encoding two glycozymes into the backbone of a baculovirus genome. The thereby generated SweetBac virus was subsequently used for the production of the human HIV anti-gp41 antibody 3D6, by integrating heavy and light chain open reading frames into the SweetBac genome. The overall expression rate, especially in the recently established Tnao38 cell line, was comparable to that of transient expression in mammalian cells. In order to evaluate the ability of SweetBac to generate mammalian-like N-glycan structures on 3D6 antibody, we performed SDS/PAGE and tested for the presence of terminal galactose using Riccinus communis agglutinin I. The mammalianised variants of 3D6 showed highly specific binding to the lectin, indicating proper functionality. To confirm these results, PNGase A released N-glycans were analyzed by MALDI-TOF-MS and shown to contain structures with mainly one or two terminal galactose residues. Since the presence of specific N-glycans has an impact on antibodies ability to exert different effector functions, we tested the binding to human Fc gamma receptor I present on U937 cells.

P10-2
Differentiation and activation of macrophages: a galectin-3 perspective

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Galectin-3 (Gal-3), a β-galactoside lectin, is mainly considered to advance inflammation: it triggers/promotes monocyte respiratory burst, acts as a monocyte (Mc)/macrophage (Mf) chemoattractant and promotes the survival of inflammatory cells. Depending on environmental cues, Mc/Mf exhibit diverse biological characteristics, but two main subtypes, classically (M1) and alternatively (M2) differentiated and activated Mf have been recognized. This study explores the expression of Gal-3 in the physiology of these cells. Monocytes from healthy blood donors were differentiated to M1 or M2 cells using macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), respectively. Macrophages were then activated classically by IFN-γ and LPS, or alternatively, using IL-4/IL-10 to generate M2a/c cells. Gene and protein expression levels of intra- and extracellular Gal-3 were investigated by qRT-PCR, Western-blot, flow cytometry, imunoprecipitation and ELISA, while surface Gal-3 receptor expression was analyzed by flow cytometry. Differentiation of Mc and classical/alternative Mf activation was followed by marked changes of Gal-3 expression and proteolitic cleavage. Furthermore, its expression and secretion were tightly regulated and significantly differed among distinctly differentiated and activated Mf. Interestingly, considerable differences in Gal-3 expression profiles were observed among the same Mf subtypes, obtained from different donors. Upon activation by IFN-γ and LPS, M1 (but not M2) macrophages diverged into distinct populations with respect to membrane Gal-3 expression. Human Mc had a high amount of free Gal-3 receptors, while on activated Mf the receptors were fully saturated. Specific expression and secretion patterns of Gal-3 in differentiated and activated Mf contribute to better understanding of its role in these cells, and provide an important insight into macrophage biological characteristics.

P10r-3
Lyn-coupled LacCer-enriched lipid rafts in neutrophilis: a possible organization

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Glycosphingolipids (GSL) are membrane components consisting in a hydrophobic ceramide (Cer) and in a hydrophilic sugar moieties. They cluster to form GSL-enriched domains on cellular plasma membrane (lipid rafts, caveolar domains, and glycosynapses) providing a microenvironment within the plasma membrane for reciprocal interactions between lipids and proteins. Biochemical analyses have demonstrated that GSL-enriched microdomains contain several kinds of transducer molecules, especially membrane-anchored signal transduction molecules, such as Src family kinases. It has been speculated that GSLs are closely associated with cell processes like differentiation, proliferation and phagocytosis, but there are quite few evidences that GSLs by themselves directly mediate signal transductions, which lead to cell functions. Lactosylceramide (LacCer), a neutral GSL, is abundantly expressed on human neutrophils, and specifically recognizes several pathogenic microorganisms. We previously demonstrated that LacCer forms detergent resistant domains (DRM) coupled with the Src family kinase Lyn, on the PM. Ligand binding to LacCer activates Lyn, resulting in neutrophil functions, such as superoxide generation and migration. The presence of a LacCer molecular species with Cer containing a very long fatty acid chain is necessary for the association of Lyn with LacCer-enriched DRM and LacCer-mediated functions. Lyn molecules are attached to the inner leaflet while LacCer is component of the outer leaflet of membrane bilayer. So, how does LacCer interact with signal transducer molecules? To explore the molecular mechanism responsible for the association between fatty acid chains of LacCer and Lyn in LacCer-enriched DRM, we prepared analogues of C24-LacCer and C16-LacCer containing tritium and photoactivable group. Similar analogues of glycosphingolipids have been successfully used in the past to study sphingolipid-protein interactions. We found that (i) LacCer

is immunoprecipitated with Lyn in DRM fraction, when cells are loaded with C24-LacCer analogue, but not with C16-LacCer analogue; (ii) Lyn has a direct interaction with LacCer in DRM fraction, because Lyn became radiolabeled after the loading with C24-LacCer analogue followed by illumination. For the first time, at the best of our knowledge, we show a direct connection, across the plasma membrane, between GSLs and palmitoylated proteins. These results suggest that LacCer with a long fatty acid chain in Cer moiety could be the key-player of the transduction of information across the plasma membrane, modulating membrane interdigitation, through the long acyl chain, and forming specific plasma membrane microdomains.

P10-4
Extraction by supercritical carbon dioxide ($scCO_2$) and analysis of polar lipids from bifidobacteria

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This study describes an efficient isolation procedure for extraction of polar lipids from two strains of bifidobacteria – *B. longum* and *B. angulatum*. We first compared the lipid profiles of two strains of bifidobacteria and showed that the strains of the same genus may differ by lipid compositions of the cell wall. The polar lipids were isolated using the following conditions: pressure 250 mbar, temperature 45°C, CO_2 flow rate 5 g/min, hydromethanolic co-solvent concentration 10% (v/v). In the lipid extracts the carbohydrate moiety of glycolipids and in phospholipids phosphorus moiety were quantified and compared to the reference material isolated by classical extraction. Thrice the amount of glycolipids and phospholipids in comparison with classical method was found in the lipid extract from *B. longum* obtained with $scCO_2$, and twice the amount of more – from *B. angulatum*. By TLC were identified different glycolipid profiles of these strains, four glycolipids were detected from *B. longum* and seven glycolipids from *B. angulatum*. From 5 g of biomass during prolonged fractionated extraction (12 hours) 2.80 and 3.11 mg of glycolipids, 1.86 and 1.88 mg of phospholipids from *B. longum* and *B. angulatum* was obtained, respectively. ELISA of polar lipids from *B. longum* and *B. angulatum* showed that the glycolipid fractions are more immunoreactive compared with phospholipids – in the case of the glycolipid fractions marked reaction with the serum of healthy rabbits began from 1600-fold dilution, with phospholipids – from 800-fold dilution.

P10-5
Glycoconjugates of bifidobacteria and lactic acid bacteria deposited in Belarus Collection of Microorganisms

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Currently, over 1300 microbial strains are maintained at Belarus Collection of Microorganisms. Some of these strains are used in manufacturing: enzyme preparations, in ensilage plant substrates, therapeutic compositions enhancing immunity of humans and animals, biological agents to control plant pathogens, preparation for bioremediation of waste. Introduction of chemical and biochemical methods for characterization of polysacharides, phospholipids and glycolipids, as chemotaxonomic markers of microorganisms and the components of therapeutic products is one of the trends in the collection. Thus, it is utmost important to isolate, identify and characterize these biologically active polar lipids from bifidobacteria and lactic acid bacteria, by using supercritical carbon dioxide extraction that is a very effective and environmentally friendly method. The major components of interest in our studies were glycolipids. The highest glycolipids yield was obtained from dry bacteria biomass at process parameters: 250 bar, 40°C, CO_2 flow rate 10 g/min, hydromethanolic co-solvent 9.3–10.0% (methanol-water 9:1 v/v). The TLC-analysis revealed the presence of two major glycolipid fractions identical to those obtained with classical organic solvent extraction. Glycolipids were subjected to acid methanolysis and fatty acid composition was determined by GLC-analysis. Palmitic, myristic and erucic acid were the predominant fatty acids. The carbohydrate moiety was analyzed after acidic hydrolysis using GLC. Glucose and galactose were the major shugars of the isolated glycolipids. Glycolipids with high immunoreactivity were identified by ELISA. The results indicate that supercritical carbon dioxide does not alter structure of glycolipids. In prospect, the supercritical carbon dioxide technology may be combined with metabolic engineering and immunological studies in manufacturing of highly effective therapeutic products.

P10-6
Adhesive and migratory behaviour of pancreatic adenocarcinoma Capan-1 cells is regulated by changes in α2β1integrin sialic acid determinants

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Integrins are membrane glycoproteins involved in cell adhesion to extracellular matrix proteins. They activate intracellular signalling processes, such as the ones that involve focal adhesion kinase (FAK). In cancer, up-regulation of integrins and FAK signalling promote migration and metastasis of tumour cells. The purpose of the present study is to evaluate the effect of sialyl-Lewis x (SLe x) overexpression in regulating the function of α2β1integrin.

For that, we use as a model the pancreatic adenocarcinoma cell line Capan-1, which has medium level of endogenous α2,3-sialyltransferase ST3Gal III and expresses SLe^x, and its stable ST3Gal III transfectant C31, which expresses the double of SLe^x and almost four times less of α2,6-sialic acid structures than Capan-1. Blocking Capan-1 cells with antibodies against integrin subunits showed that more than 90% of the adhesion to type 1 collagen was via α2β1integrin. *In vitro* adhesion to collagen and migration assays showed that C31 had lower adhesion to collagen and increased migration compared to Capan-1. The glycosylation of α2β1 integrin in both cell lines was examined by immunoprecipitation and further blotting with anti-SLe^x or *Sambuccus nigra* lectin. The results showed that α2β1integrin from C31 had increased SLe^x and decreased α2,6-sialic structures compared to the one from Capan-1 These glycosylation changes may contribute to a greater activation of the signaling pathway initiated by phosphorylation of FAK at tyrosine 397, which was higher phosphorylated in C31 and would explain C31 greater migratory capacity.

In summary, α2β1 integrin functionality is finely regulated by a shift in its sialic acid content and linkage. This strategy could supply the tumour cell with a powerful tool to regulate its adhesive and migratory capacities throughout the tumour progression steps.

P10-7
On the study of the structure and dynamics of a carbohydrate of great biological importance: heparin

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Extracellular modulation of phenotype is an emerging paradigm in the current postgenomics age of molecular and cell biology. Glycosaminoglycans (GAGs) are primary components of the cell surface and the cell–extracellular matrix (ECM) interface. Advances in the technology to analyze GAGs and in whole-organism genetics have led to a dramatic increase in the known important biological role of these complex polysaccharides. Due to their ubiquitous distribution at the cell-ECM interface, GAGs interact with numerous proteins and modulate their activity, thus being involved in fundamental biological processes such as cell growth and development.

The glycosaminoglycan heparin shows particular conformational plasticities due to the flexibility of its L-iduronic acid constituents, which affords it with remarkable protein adaptability. These residues are in equilibrium between different conformations ($^1C_4$, ^2S_O, and $^4C_1$), and their relative proportion of conformers is a function of sulphation pattern and sequence. Thus, in the framework of a project aimed to explore the molecular bases of the selectivity in the interactions of GAGs with biologically interesting heparin binding proteins using synthetic oligosaccharides, we have synthesized a library of trisaccharides heparin like models, highly pure and structurally perfectly defined.

In addition to their suitability for the interaction studies, this library represents the minimum structural features around iduronate residues within GAG chains, in which the different possible substitution patterns are systematically varied. On the other hand, their small sizes allow to determine distances from NOEs with high accuracy as well as to measure very precise $^3J_{H\text{-}H}$ values. Together with NMR experiments at variable temperature, these trisaccharides have been studied by molecular dynamics simulations, with and without experimental constraints, in explicit water.

P10-8
Cell-autonomous heparanase modulates self-renewal and migration in bone marrow-derived mesenchymal stem cells

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent stem cells and are potentially applicable in wide variety of pathological conditions. However, the niche microenvironment for BM-MSCs maintanence has not been clearly characterized. Accumulating evidence implies that heparan sulfate glycosaminoglycans and heapranase (HPSE1) modulate the self-renew and differentiation of BM-MSCs. To clarify the physiological role of HPSE1 in the stem cell properties of BM-MSCs, we inhibit the enzymatic activity of HPSE1 in isolated BM-MSCs. Quantitative RT-PCR detected HPSE1 mRNA expression in low levels in isolated BM-MSCs. While the expression levels of HPSE1 fluctuate during *in vitro* tri-lineage differentiations, enzymatic inhibition does not affect either osteogenic or adipogenic differentiation. However, both cell proliferation counts and CFU assays are decreased when HPSE1 is enzymatically inhibited. Moreover, HPSE1 inhibited groups showed better mobility than native BM-MSCs in migratory assay. Our findings indicated that self-renewal and migration of BM-MSCs are modulated by autonomous HPSE1.

P10-9
Can galectin-3 distinguish two types of classically activated macrophages?

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As they migrate from the blood, monocytes differentiate into macrophages (Mf) and populate nearby tissues. Mf can be activated into a distinct pro-inflammatory phenotype known as classical (M1) macrophages, which promote a TH1 response, killing of intracellular pathogens and inflammatory mediator release. During this process, the expression of thousands of proteins is modulated, including a pro-inflammatory lectin, galectin-3 (Gal-3). This β-galactose-binding galectin is a key regulator of both innate and acquired immunity, and participates in all phases of the inflammatory response. Gal-3 promotes monocyte respiratory burst, macrophage chemotaxis and phagocytosis.

The aim of this study was to explore the surface expression Gal-3 during classical Mf activation by IFN-γ/LPS. Mo were isolated from healthy blood donors and differentiated into two distinct subtypes using granulocyte-macrophage (GM-) or macrophage colony-stimulating factor (M-CSF). Both Mf subtypes were activated by IFN-γ/LPS, and the surface Gal-3 expression was analyzed by flow cytometry. 24 hour of classical activation of Mf obtained by GM-CSF treatment yielded 2 distinct populations of M1 cells regarding the surface Gal-3 expression ($M1^{G3\text{-}hi}$ and $M1^{G3\text{-}lo}$). The observed phenotype remained stable for up to 72 hour. Such Gal-3 expression profile was not detected upon LPS/IFN-γ activation of Mf obtained by M-CSF treatment. It seems that GM-CSF-induced differentiation is essential for sub-dividing of Mf into two distinct populations regarding Gal-3 expression, upon LPS/IFN-γ activation.

Specific Gal-3 membrane expression profile could contribute to understanding of its roles in the physiology of M1 macrophages, but also provide a new insight into the sub-classification of these cells and their biological roles.

P10-10
Stage-specific expression and antigenicity of glycoprotein glycans isolated from the human liver fluke, *Opisthorchis viverrini*

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Infection by *Opisthorchis viverrini* (liver fluke) is a major public health problem in Southeast Asia, resulting in hepatobiliary disease and cholangiocarcinoma. Fluke surface glycoconjugates are prominently presented to the host, thereby constituting a crucial immunologic interface that can determine the parasite's success in establishing infection. We have investigated *N*- and *O*-linked glycoprotein glycan profiles of the infective metacercarial stage and of the mature adult by nanospray ionization-linear ion trap mass spectrometry (NSI-MS^n) Glycan immunogenicity was investigated by immunobloting with serum from infected humans. Metacercaria and adult parasites exhibit similar glycan diversity, although the prevalence of individual glycans and glycan classes varies by stage. The N-glycans of the metacercaria are mostly high mannose and monofucosylated, truncated-type oligosaccharides (62.7%), with the remainder being complex and hybrid type (37.3%). The N-linked glycan profile of the adult is also dominated by high mannose and monofucosylated, truncated-type oligosaccharides (80.0%), with a smaller contribution from complex and hybrid type (20.0%). At both stages, complex and hybrid type glycans are detected as mono-, bi-, tri-, or tetra-antennary structures. In metacercaria and adults, *O*-linked glycans are detected as mono- to pentasaccharides. The mucin type core 1 structure, Galβ1-3GalNAc, predominates in both stages but is less prevalent in the adult than in the metacercaria. Immunogenic recognition of liver fluke glycoproteins is reduced after deglycosylation but infected human serum was unable to recognize glycans released from peptides. Therefore, the most potent liver fluke antigenic epitopes are mixed determinants, comprised of glycan and structural elements of associated polypeptide backbones.

P10-11
Proteomic and glycoproteomic research of recombinant fungal α-*N*-acetylgalactosaminidase from *Aspergillus niger*

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Introduction: Alpha-*N*-acetylgalactosaminidases (α-NAGA) are exoglycosidases specific for the hydrolysis of terminal α-linked N-acetylgalactosamine in various sugar chains. The enzyme from *A. niger* has certain unique properties, therefore it was chosen for further investigations. These properties can find a use for the enzymatic synthesis of various carbohydrate structures and for transformation of the red blood cell group A to the group of H (0), the universal donor.
Results: The cloned DNA is 1450 bp long, and the deduced amino acid sequence (480 amino acid residues with molecular mass 54.814 kDa) is almost identical to that of purified enzyme (as determined by SDS/PAGE). For investigation of the molecular weight of the recombinant α-NAGA, gel filtration, analytical ultracentrifugation, and dynamic light scattering were used. The wild type and recombinant α-NAGA produced in nonglycoengineered and humanized S.cerevisiae W303 exhibited value Km to be 0.5588 mmol/l in 50 mM citrate-phosphate buffer (pH = 3.5) at 37°C. The purified enzymes have different optimal, different optimal temperature and temperature stability of individual α-NAGAs were found.
Conclusions: The α-NAGA from A.niger CCIM K2 was expressed in different glycoengineered strains of *S. cerevisiae*. The optimal conditions for production and purification were found. The basic biochemical and enzymatic properties of individual α-NAGAs were investigated. The analysis of α-NAGAs with mutated N glycosylation sites is now a work in progress. The method for transformation of erythrocytes type A to the type H(0) is also currently being investigated.
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P10-12
Overexpression of serglycin promotes breast cancer cell aggressiveness

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Serglycin proteoglycan is expressed by malignant cells and is implicated in metastasis. In certain tumors it protects tumor cells from complement system attack. Although the expression of proteoglycans in breast cancer has been extensively studied there are no published data on the expression of serglycin. In our study the expression of serglycin at transcriptional levels in cancer cell lines showing different metastatic potential was examined by RT-PCR analysis. The high expression of serglycin in the most aggressive cancer cells MDA-MB-231 was revealed compared to the low expression in the low metastatic MDA-MB-468 cells and in the non metastatic MCF-7 cells. Moreover, serglycin was secreted only by MDA-MB-231 cells. It exhibited a strong filamentous cytoplasmic staining in these cells and was also present in filopodia and membrane protrusions. The expression of serglycin by breast cancer cells was also confirmed by immunohistochemistry in patients' material. Stable transfection of MCF-7 cells with serglycin induced their proliferation, anchorage-indepented growth and migration. Interestingly, overexpression of serglycin lacking

the glycosaminoglycan-bearing domain failed to promote these cellular functions suggesting that glycanation is a pre-requisite for its tumor-promoting properties. Our findings suggest that serglycin promotes a more aggressive cancer cell phenotype

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P10-13
Galectin-8 enhances B cell antigen presentation

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Activation of B lymphocytes in response to pathogens is a key step of the adaptive immune response, which is required for the production of high affinity antibodies and the generation of long-term memory. In lymph nodes (LNs), it is initiated by the engagement of the B cell receptor (BCR) with antigens (Ag) immobilized at the surface of neighboring cells and leads to the formation of a synapse that promotes Ag uptake for presentation onto MHCII molecules. Upon synapse formation, MHCII-containing lysosomes are recruited to the BCR-Ag synaptic interface where they locally undergo exocytosis, allowing synapse acidification and the extracellular release of hydrolases that promote the extraction of the immobilized Ag. Galectins are secreted through unconventional mechanisms and once secreted they can cross-link cell surface glycoproteins. This property enables them to modulate a wide range of processes such as cell growth, apoptosis and migration. Within the immune system galectins are significantly up-regulated by activated macrophages, NK, T and B cells and can influence effector functions by modulating signaling and cytokine secretion. Galectin-8 (Gal-8) contributes to B cell differentiation into plasma cells and has been implicated in autoimmunity. In this work, we analyzed the impact of Gal-8 on B cell synapse and show that it enhances the Ag processing and presentation in B cells. Our results suggest that in addition to BCR ligands, the presence of Gal-8 triggers a faster recruitment of lysosomes to the B cell synapse, thus promoting a more efficient extraction of immobilized Ag. This study provides novel insight on how extracellular cues can tune the ability of B cells to respond to Ag and ultimately determine the outcome of their fate *in vivo*.

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P10-14
Glycomimetic ligands of DC-SIGN: detailed characterization of their binding modes and structural requirements for selectivity

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DC-SIGN is a C-type lectin mainly expressed by immature dendritic cells (DCs), located in the skin or peripheral mucosal tissues. It presents a Carbohydrate Recognition Domain (CRD) at the C-terminus, responsible for the recognition of a broad spectrum of pathogens. Its internalization pathway is exploited by HIV-1 to facilitate trans-infections of T cells and to invade the host immune system. On the contrary, Langerin, another C-type lectin, blocks HIV transmission by its internalization in specific organelles of Langerhans cells. Thus, approaches aiming to inhibit DC-SIGN without blocking Langerin, represent attractive strategies and, certainly, a rational design of glycomimetic drugs requires a detailed characterization of the ligand binding mode. High resolution of DC-SIGN/ligands complex structures have been solved by crystallography, but additional approaches are crucial to get a deeper structural insight of the carbohydrate/lectin binding modes, even more in the case of DC-SIGN, for which previous studies demonstrated a recognition of some ligands in a multimodal fashion (1–2). In this work we have combined X-ray results with NMR techniques in solution [mainly TR-NOESY and STD NMR (3)] and computational methods [CORCEMA-ST protocol (4)] to obtain structural information at atomic resolution on the ligands binding modes.

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P10r-15
Lectin histochemistry of the gills and intestine of the turbot (*Psetta maxima*) after exposure to BDE-47

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The mucus secreted by the epithelium lining the gills and intestine of fishes contains glycoconjugates involved in several biological functions, such as defence against pollutants. Previous studies have revealed that the exposure of turbots to a common environmental contaminant (BDE-47) induces mucus hypersecretion due to hyperplasia and hypertrophy of the gills and intestinal goblet cells.

Here we used lectin histochemistry to determine changes in the composition and distribution of carbohydrates in the gills and intestine of juvenile turbots after exposure to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) for 15 days.

The experimental design involved four treatments: control, a polar aprotic solvent (DMSO 0.1‰) and two doses of BDE-47, dose 1 (0.03 μg/l) and dose 2 (0.3 μg/l). Samples of gills and intestine were fixed in an aqueous Bouin's solution and embedded in paraffin. Each tissue section was incubated with 7 biotinylated lectins, which identify specific terminal sugar residues of glycoconjugates. The results were evaluated semi-quantitatively on the basis of the relative staining intensity described by two independent observers.

Results showed that BDE-47 altered the glycosilation pattern of the mucin secretion in the gills by inducing the presence of mannose and/or glucose, *N*-acetyl-D-galactosamine and β-D-galactose and α-D-Gal(1-3)*N*-acetylgalactosamine. However, no compositional changes were observed in the glycoconjugates of the gill epithelium. In contrast, the intestine lining cells did not react to the toxicant and neither the mucin glycoproteins nor glycoconjugates altered their carbohydrate composition.

In conclusion, BDE-47 exposure produces effects on fish gills by enhancing the production of glucidic residues involved in toxicant defensive responses.

P10-16
Glycod'Express™ and YAC-Express™: two innovative technologies developed for N-glycosylation humanization and production of therapeutic recombinant glycoproteins in *Saccharomyces cerevisiae*

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The production of therapeutic recombinant glycoproteins deals with three main issues: cost, production capacities and glycosylation. Nowadays, such proteins are derived from various complex expression systems (CHO, bacteria etc…); the processes related to those production hosts are time-consuming, expensive or the question of post-translational modifications (as glycosylation) control is still unresolved. There is a need to find an alternative, while maintaining high quality level: the new system must be able to add complex N-glycan structures to the protein of interest. Developed in several strains of *Saccharomyces cerevisiae*, Glycod'Express™ and YAC-Express™ are innovative technologies that allow production of therapeutic recombinant glycoproteins with humanized and homogeneous N-glycan moieties. We show which mannosyltransferases involved in host N-glycosylation have to be deleted to obtain more than 70% of homogeneity in glycan structures. The methodology developed to select the optimal fusion between a heterologous glyco-enzyme and a localization sequence is also illustrated. As some nucleotide-sugar donors are missing in the budding yeast secretory pathway, an innovative method for GDP-fucose synthesis is described. The sequential engineering of the various strains allow developing the Glycod'Express™ and the YAC-Express™ technologies that are finally presented.

P10-17
NMR spectroscopy to study the interactions of Langerin with sulfated glycosaminoglycans

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Epidermal Langerhans cells (LCs) constitute a subset of dendritic cells (a sentinel immunity cell lineage) that express langerin, a C-type lectin which is a crucial component of Birbeck granules (BGs), subdomains of the endosomal compartment specific to LCs (1). Langerin acts as a pathogen receptor by binding to surface glycoconjugates of a number of microorganisms (fungi, mycobacteria, viruses). Notably, langerin can prevent transmission of HIV from LCs to T cells by mediating internalization into BGs and degradation of the virus (2). Within the C-type family of lectins, langerin seems to be a unique receptor as it has shown to have dual specificity, being able to recognize both, mannosylated and sulfated glycans, via a single C-type carbohydrate recognition domain (CRD) (3,4).

In this work we are interested in the characterization in structural terms of the second specifity of langerin; that for sulfated glycans. We have applied NMR spectroscopic techniques to study the interactions of sulfated glycosaminoglycan (GAG) ligands to the extracellular domain (ECD) of langerin in solution. We have paid particular attention to the effect of the different sulfation patterns of the ligands, as well to the role of the divalent cation, on the interactions. We demonstrate that STD NMR and transferred-NOE experiments can be optimized and successfully carried out on these highly charged systems, from which the binding epitopes and the bioactive conformations can be obtained. Our investigations highlight differences in binding modes depending on the length of the GAG ligand.

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P10r-18
Study of the heparanome in ascending colon adenocarcinoma with nodal metastases

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Colon cancer is the third most common malignancy in men and the second in women in the developed world, being the predominant form the invasive adenocarcinoma. Glycosaminoglycans (GAGs) are linear heteropolysaccharides which have a complex structure. The heparan sulfate (HS) GAG is a complex structure with functional domains capable of binding selectively a broad range of ligands. In the cells, the HS appears as part of heparan sulfate proteoglycans (HSPGs), which are essential components of the cells and of the extracellular matrix. The HSPGs are involved in a wide variety of essential physiological effects, changes being described in numerous pathologies, particularly in different tumors. Often, chains of chondroitin sulfate (CS) appear linked to the HSPG structures. The aim of this study was the analysis of the differential expression of all genes involved in HSPGs biosynthesis in ascending colon infiltrating adenocarcinoma with nodal metastases. Alterations in the genes of HS and CS occur at specific stages of the biosynthesis of these GAGs. We detected altered expression in 15 out of 63 genes studied. Specifically, we observed overexpression of two of the four genes that form the binding tetrasaccharide core protein as well as the HS epimerase. Conversely, a quantitatively significative decrease of CS epimerase was observed. No changes were observed at the level of polymerases; however it was evidenced a subexpression of 6OST-1, involved in HS modification, and three of the enzymes that modify CS at the C4, CS4ST-1, CS4ST-2 and DS4ST. With regard to HSPGs core proteins, we determined significant decreases of syndecans 2 and 3 and glypican 1 at the

level of cell surface. Moreover, in the extracellular matrix appeared underexpressed perlecan and collagen XVIII. On the other hand, serglycine, mainly intracellular, also showed significant subexpression.

P10-19
Alpha-L-fucosidase expression in colorectal tumors

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Changes in fucosylation of the cell surface glycoconjugates have been reported in colorectal tumors and appear to be of great significance for the metastatic potential of tumoral cells. The glycosidase alpha-L-fucosidase (EC 3.2.1.51) catalyzes the hydrolytic elimination of fucose residues from glycoconjugates, and we have demonstrated that its activity is decreased in colorectal tumors. The aim of this study was to determine the expression of FUCA1, the gene encoding tissue alpha-L-fucosidase (AFU), in tumor and normal mucosa from colorectal cancer patients.

In this work tumor biopsies and their corresponding healthy mucosa from 32 patients with colorectal cancer at different Dukes′ stages were included. Total RNA was isolated and reverse transcribed into complementary DNA (cDNA). cDNA was used to determine FUCA1 expression by real-time quantitative PCR (qPCR). To quantify the expression of FUCA1 it is necessary to normalize it by stably expressed internal reference genes. Therefore 5 candidate genes described in the literature (B2M, GAPDH, HPRT1, PPIA, RPLP0) were quantified and evaluated. Among them applying the softwares geNorm and NormFinder we found that RPLP0 and HPRT1 were the most suitable reference genes for colorectal tissues. Normalized FUCA1 gene expression in tumor and mucosa samples from each patient was compared and a statistically significant decrease was found in tumors. In order to validate these results the expression of AFU protein was analyzed by Western blot using a specific anti-FUCA1 antibody. As expected, our results showed that AFU protein expression was reduced in tumors in comparison with normal mucosa

This study proves that the lower expression of alpha-L-fucosidase protein in colorectal tumors is due to a decreased expression of FUCA1.

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P10-20
Production of *Aspergillus niger* β-mannosidase in *Pichia pastoris*

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β-Mannosidase (EC.3.2.1.25) is an exoglycosidase specific for the hydrolysis of terminal β-linked mannosides in various sugar chains. This enzyme is essential for the complete hydrolysis of β-mannans to mannose.

A number of β-mannosidases have been prepared from various sources but none of them expressed in yeast. In this study, we report the first fungal *A. niger* β-mannosidase to be successfully cloned, sequenced and expressed in the yeast *P. pastoris*. The β-mannosidase gene contained a 2799-bp open reading frame that encoded a mature protein of 933 amino acids. The wild and recombinant proteins were purified to apparent homogeneity and biochemically characterized (K_M 0.28 and 0.44 mmol/l for *p*-nitrophenyl β-D-mannopyranoside, p*I* 4.2 and 4.0, and their optimal enzyme activities were achieved at pH 4.5 and 5.5 and 65°C, respectively).

The molecular mass of the recombinant β-mannosidase was ~158 kDa as determined by SDS/PAGE, which was higher than wild β-mannosidase (130 kDa).

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P10-21
Fungal α-L-rhamnosidase – properties and crystallization

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α-L-Rhamnosidase (EC 3.2.1.40) is a biotechnologically important enzyme used for derhamnosylation of many natural compounds. Only a few reports on the isolation, cloning and expression of the gene encoding α-L-rhamnosidase are available. The crystal structure of only single α-L-rhamnosidase, RhaB from *Bacillus* sp. GL1 (1), has been determined, but no crystal structure of a fungal α-L-rhamnosidase has been reported so far. Fungal α-L-rhamnosidase from *Aspergillus terreus* is used for the conversion of rutin to isoquercitrin, a pharmacologically significant flavonoid (2). *Aspergillus terreus* α-L-rhamnosidase shows unique biochemical capabilities, catalyzing reactions proceeding under extreme conditions, such as 70°C and pH 8.0 for 24 hours. This enzyme was also investigated on molecular-genetic level, its gene rhaD was sequenced and cloned for preparation of the recombinant enzyme. The extracellular overexpression was reached (3). Other attractive ability of this enzyme is reverse hydrolysis – production of new rhamnosides. For structural studies, the protein was purified from its natural source. Crystallization conditions were found through extensive screening followed by optimization. The α-L-rhamnosidase from *Aspergillus terreus* was succesfully crystallized and the best crystal diffracted to resolution 1.9 Å.

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P10-22
Macroautophagy-lysosomal system (mals) in gaucher patients carrying L444P and N370S mutations

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Gaucher disease (GD) is caused by defects in the activity of the lysosomal enzyme, glucocerebrosidase (GCase). The misfolded GCases in the endoplasmic reticulum (ER) in Gaucher patients are expected to activate the ER activated autophagy (ERAA). Additionally the glucosylceramide that accumulates in the lysosomes of the Gaucher patients may negatively impact the turnover rate of substrates, including those brought in through the autophagic pathway (the flux). We aimed to determine the effects on MALS of glucosylceramide storage, by monitoring of LC3B-I, LC3B-II levels and the general rate of lysosomal proteolysis using DQ-BSA in clinically different types of Gaucher patients fibroblasts carrying L444P and N370S mutations. LC3B-II is the only protein marker that is reliably associated with complete autophagosomes and phagophores, and is eventually turned-over in the autolysosome and/or lysosome. Elevated levels of LC3B-II in Gaucher fibroblasts were observed, demonstrating an increase in the number of autophagosomes. This could occur through the induction of autophagy and/or a decrease in the rate of lysosomal turnover of LC3B-II (autophagic flux). To determine the efficiency of lysosomal proteolysis, fluorescent DQ-BSA was added to the media of live Gaucher fibroblasts and its rate of turnover analyzed with high throughput instrument Cellomics KSR, which is capable of resolving spatially and temporally separated fluorescent labels to quantitatively measure multiple features simultaneously. Turnover of the DQ-BSA were found significantly lower in the Gaucher fibroblasts compared to wild type. Our results revealed that the clearance of aggregated proteins through the autophagic pathway is inhibited as a consequence of a decreased ability of lysosomes to process the contents of autophagosomes in L444P and N370S mutations, more significant in type II acute neuronopathic form and correlated with the severity of phenotype. It follows that the apparent increase in the number of autophagosomes is due to a decrease in autophagic flux in L444P and N370S mutations.

P10-23
A Delta och1 *Saccharomyces cerevisiae* developed for heterologous production of therapeutic glycoproteins

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Microorganisms such as the yeast *Saccharomyces cerevisiae* are widely used cell factories for the production of heterologous proteins of medical or industrial interest; they also often are the expression system of choice when manufacturing costs are of primary concern. Their rapid growth, microbiological safety, and high-density fermentation in simplified medium have a high impact, particularly in the large-scale industrial production of proteins, where secretion in the supernatant is important for simplifying the downstream purification process. Yeasts share, with mammals, a secretory pathway that allows protein glycosylation: the sugar chains are composed of mannose polymers, which are somewhat antigenic in humans and so cleared by mannose-specific receptors or lectins. In the Golgi apparatus, critical mannosyltransferases are involved in *S. cerevisiae* N-glycosylation; Och1 is responsible for the initiation of the outer chain of up to 200 mannoses. Therefore glycan remodeling to human glycans is required for the production of therapeutic glycoproteins in yeast. We developed *S. cerevisiae* strains deleted for the och1 gene, and we were successful in producing heterologous glycoproteins with mammalian-type high-mannose N-linked oligosaccharide. We are now optimizing the secretion, by this yeast strain, of proteins of interest. This is the first step in the humanization of the N-glycosylation.

P10-24
Sialic acid as inflammation marker, in serum and tissues in experimental hypo – and hyperthyroidism

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Sialic acid (SA), a family of acetylated derivatives of neuraminic acid, is widely distributed in mammals. It is the most common terminal carbohydrate residue in the glycan moiety of glycoproteins. It plays an important role mainly in the cell-cell interaction. SA has also been proposed as a marker of acute phase response in different diseases.The previous studies have shown that oxidative stress can cause desialylation of glycoproteins. The biological role of thyroid hormones is to maintain the normal energy level, growth, temperature and development. Disorders of thyroid gland are among the most frequent endocrine disorders. The major target organ for thyroid hormones is liver and it also affects other tissues. It has been suggested that enhanced sialylation of glycoproteins occures during hypo- and hyperthyroidism. In this study, hypothyroidism and hyperthyroidism were induced in adult female Wistar Albino rats by feeding methimazole and L-thyroxine in diet respectively. SA levels of serum and tissue samples such as liver, kidney and brain were determined by the method of Warren. SA levels were markedly increased in all experimental groups in comparison to the control group. The increased content of serum and tissue SA in hypo- and hyperthyroid rats might possibly have come from disruptions of membranes because of oxidative stress. The effect of thyroid hormones can significantly change the content of SA. In determining of mechanism of action further studies are needed to be done.

P10-25
Decrease of O-beta-*N*-acetylglucosaminidase expression in murine skeletal muscle cell atrophy

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Background: Protein O-linked beta-*N*-acetylglucosaminylation (O-GlcNAc) is a regulatory post-translational event, which consists of the attachment of O-GlcNAc to serine or threonine residues, the same site of phosphorylation, modulated by two O-GlcNAc regulatory enzymes: O-GlcNAc-transferase (OGT) and O-beta-*N*-acetylglucosaminidase (OGA). O-GlcNAc plays a crucial role in many cellular processes and is deeply related to skeletal muscle development and physiological function. Muscle atrophy is a common complication of many diseases, some of which correlate with high cellular O-GlcNAc levels, such as

diabetes. However, the relation between OGT and OGA expression levels during muscle wasting is still not completely defined. In order to better investigate this point, in particular regarding the OGA role, we employed an *in vitro* model of myotubes atrophy.

Methods: To induce atrophy, murine skeletal myotubes (C2C12) were treated with 10 μM dexamethasone (dex) for 48 hour. Expression of the atrophy-related ubiquitin-protein ligases, Atrogin-1 and MuRF-1 was determined by real time PCR. In atrophic and control-C2C12 cells (treated with 0.1% v/v EtOH) O-GlcNAc, Ser-Phosphorylation and OGA expression levels were evaluated by immunoblotting and real time-PCR.

Results: Treatment of C2C12 with dex for 48h increased Atrogin-1 and MuRF-1 expression by 4 and 2 times respectively. In this condition we observed that O-GlcNAc protein level increased of 56% in atrophic C2C12 (particularly in the range of 60–30 kDa), on the contrary Ser-phosphorylation protein level decreased of 33%. Assay of OGA expression by real time PCR showed a decrease of about 58% after dex treatment, whereas no significant changes of OGT expression were found.According to real time PCR, immunoblotting confirmed the decrease of OGA (about 34%) in atrophic myotubes.

Conclusions: Our data clearly highlight the main role of OGA in the muscle atrophy mechanism and emphasize the relevance of OGA modulation in skeletal muscle.

P10-26
Immunosuppressive drugs influence glycosylation of human immune cells in MLR culture

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Posttranslational glycosylation contributes to multiple biological function of immune cells relevant for adaptive and innate immunity. Interactions by glycan component of immune cell receptors are important after organ transplantation. An effect of immunosuppressive drugs, crucial for the successful transplantation of organs, on glycosylation of immune cells is not known.

We have analyzed glycosylation profiles of mononuclear cells (MNCs) treated with immunosuppressive drugs. MNCs, freshly isolated from two healthy donors, were activated in MLR culture in the presence of the single drug cyclosporine A (CsA), rapamune (RAPA), tacrolimus (Tac) or double CaS/RAPA, Tac/RAPA. The results indicate on changes in glycosylation profile of MNCs treated with CsA and Tac in comparison to untreated cells. Interestingly, co-treating MNCs with RAPA/CaS and RAPA/Tac did not induce any changes of glycosylation. We observed differences in reaction with fucose recognizing lectins (AAA, UEA and TPA) and high mannose- (GNA), beta1,4 linked GlcNAc- (DSA) and GlcNAc oligomes- (LEL) binding lectins. The changes concern mainly two bands corresponding to 50 and 45 kDa proteins. The results obtained by lectin blotting were confirmed by lectin staining in flow cytometry. Our analysis revealed that the action of CsA and Tac is much more global than it was thought so far.

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P10-27
Testing of NAG-thiazoline derivatives as new β-*N*-acetylhexosaminidase inhibitors

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β-*N*-acetylhexosaminidases (EC 3.2.1.52, CAZy GH 20 and 84) are involved in many important physiological and pathological processes. Dysfunctions in human hexosaminidases result in serious lysosomal storage disorders (Sandhoff and Tay-Sachs diseases) (1). Small molecule inhibitors of family GH20 and 84 enzymes are very important both as tools for elucidating the role of these enzymes in biological processes as well as for developing therapeutics. NAG-thiazoline, which is the one of the most effective β-Hex inhibitors, was designed as a typical hexosaminidase inhibitor scaffold, mimicking the transition state oxazoline intermediate (2). Here, we present the preparation of some NAG-thiazoline derivatives and their testing with β-*N*-acetylhexosaminidases isolated from filamentous fungi, bacteria and with human β-*N*-acetylglucosaminidase (*O*-GlcNAcase). Some of the NAG-thiazoline derivatives were prepared by lipase-catalyzed reactions (Novozym® 435; Sigma-Aldrich) and by click chemistry (Cu-AAC reaction). Surprisingly, the compounds tested displayed atypical inhibition pattern and, thus cannot be considered as competitive hexosaminidase inhibitors as the parent compound NAG-thiazoline.

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P10-28
The impact of tunicamycin on primary uveal and cutaneous melanoma cells

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The aim of this study was to evaluate the impact of tunicamycin (TM), a specific inhibitor of GlcNAc phosphotransferase, which catalyses the transfer of GlcNAc-1-P from UDP-GlcNAc to dolichol phosphate in the first step of N-glycosylation, on primary uveal and cutaneous melanoma cells. Cells from two primary uveal (92-1 and Mel202) and two primary cutaneous (FM55P and IGR-39) melanoma cell lines were subjected TM treatment for 48 hour. Next cell viability or growth rate of the tested cells as measured by trypan blue exclusion and 3[4,5-dimethyldiazol-2-yl]-2,5diphenyltetrazolium bromide (MTT) tests. The structural alterations of cell surface N-glycans were verified by flow cytometry with the use of *Phaseolus vulgaris*, *Maackia amurensis*, *Sambucus nigra*, *Galanthus nivalis* and *Datura stramonium* lectins. The percentage of cells positive for expression of Insuline Growth Factor 1 and 2 Receptors (IGF1R and IGF2R) was estimated by flow cytometry. Determination of TM induced apoptosis was performed with the use of FITC *Annexin* V *Apoptosis* Detection Kit. Microscopic examination of 92-1, Mel202 and FM55P cells and MTT test revealed significant difference between control and TM-treated cells. TM caused major changes in the structures of N-glycans in

all tested cells, reduced IGF1R surface expression in uveal melanoma cells and induced apoptosis in cell-dependent manner.

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P10-29
Computational prediction of GPI modification sites in GPI-anchored proteins

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Glycosylphosphatidylinositol (GPI) is a post-translational modification molecule which plays important roles in the vital activities of Eukaryotic cells. Each GPI binds to a singular amino acid at a site called the omega-site in soluble proteins (GPI-anchored proteins, GPI-APs) in the Endoplasmic Reticulum and is secreted on the surface of the plasma membrane. GPI-APs are related to human incurable disorders including bovine spongiform encephalopathy, Creutzfeld-Jacob disease and paroxysmal nocturnal hemoglobinuria. Thus, identification and functional analysis of GPI-APs is believed to be crucial for the understanding of vital activities of Eukaryote cells and the resolution of molecular mechanisms of human incurable disorders.

Detection methods of GPI-APs from unkown ORF sequences were developed in this study by physicochemical properties, position specific scores (PSSs) and back-propagation Artificial Neural Networks (BP-ANNs). However, the prediction of the location of omega-sites was not included in these methods. The positions of omega-sites in GPI-APs are expected to bring essential information about sequence, structure and function of mature form GPI-APs. Thus, a highly accurate approach for predicting omega-site positions was developed by PSSs and BP-ANNs.

The GPI-AP omega-site dataset was obtained from the UniProt Knowledgebase/Swiss-Prot database. As a non-omega-site dataset, sequences around A, C, D, G, N and S residues without omega-sites were extracted from GPI-AP. PSSs were calculated based on amino acid propensities around the omega-sites then were applied to BP-ANNs which consist of a three-layered structure. More than 95% of sensitivity and specificity were estimated as results of cross-validation tests for discriminating omega-sites from non-omega-sites.

P10-30
Endothelial cell migration and expression of matrix molecules are modulated by breast cancer cells

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During tumor progression, cancer cells participate in several interactions with the tumor microenvironment as well as the surrounding cells. High level of hyaluronan (HA) is often associated with malignant progression in many cancers types. The aim of the present study was to determine the cellular responses that the breast cancer cells and particularly the highly aggressive MDA-MB-231 elicit in the human umbilical vein endothelial cells (HUVEC). For this purpose two models were utilized; one involves endothelial cells culture in the presence of breast cancer cells-derived conditioned media (CM) and the other co-culture of both cell populations in a Transwell system. We found that CM from cancer cells decreases the cell migration of HUVEC cells. To further examine this functional effect we evaluated the expression of matrix molecules implicated in cell adhesion and migration. Real-Time PCR analysis showed that the gene expressions of the HA receptor CD44, the HA synthase HAS2 and the adhesion molecules VCAM-1 and ICAM-1 in HUVEC are substantially up-regulated in both culture models used. Moreover, HA levels are significantly upregulated in both models. Notably, the expression of the membrane metalloproteinase MT1-MMP by HUVEC is significantly downregulated in both culture systems. It is worth noticing that the adhesion of cancer cells is favored by the presence of HUVEC cell monolayer in comparison with plastic surface, effect that is further induced by the presence HUVEC-secreted matrix effectors. Taking into account that CD44 and VCAM/ICAM favor the adhesive properties, whereas MT1-MMP is associated with cell migration, these data suggest that factors secreted by breast cancer cells regulate in turn the expression of matrix macromolecules and cell surface receptors which are implicated in endothelial cell migration.

P10-31
Isolation, identification and biological action of hyaluronan oligomers

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Hyaluronan (HA) is a linear non-sulfated glycosaminoglycan composed of repeating disaccharide units (→ 4-D-glucuronic acid β-1,3-N-acetyl-D-glucosamine1 →) with an overall molecular weight between 100 and 5000 kDa. HA is a major constituent of the extracellular matrix (ECM) contributing to physiological processes, such as tissue remodeling as well as in pathological conditions, such as tumor progression. High molecular weight HA has been shown to inhibit angiogenesis, whereas degradation products of low molecular weight stimulate endothelial cell proliferation and migration. Moreover, oligosaccharide HA fragments have been shown to induce angiogenesis in several animal models. The aim of this project were (i) to produce in a scale-up mode hyaluronan-derived ^{4,5}-oligosaccharides, (ii) to utilize the available biochemical tools as to monitor the HA-derived molecular sized following limited enzymatic digestion with specific enzymes (chodro-/dermato-lyases), and (iii) to evaluate the motility of cancer cells and the correlation of activity with their molecular size. The identification of the obtained oligosaccharides of various sizes was performed by Fluorophore-Assisted Carbohydrate Electrophoresis (FACE) and Capillary Electrophoresis (CE). Furthermore, we purified the various Δ-oligosaccharide fractions following gel filtration chromatography on Bio-gel P-10 column. Isolated populations were evaluated for their biological action. For this purpose, we assayed their effect in the cell motility level of the highly metastatic breast cancer cell line MDA-MB-231. The isolated fractions substantially affect cell motility and their effect varied depending on their molecular size either stimulating or inhibitory. It is worth noticing that 4-/6-oligomeric saccharides abolish the observed stimulating effects of the various HA-sized fractions. The results of this study introduce a promising field for the quality control of commercially available products in respect to the HA size distribution and highlight the importance for designing and development certain HA oligomers and/or derivatives for targeting cancer cell properties.

P10-32
FX enzyme and GDP-fucose transporter expression in colorectal cancer

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An increased number in fucosylated glycans is a common feature in several tumours. It is well known that fucosylation is regulated by fucosyltransferases, the GDP-fucose synthetic pathway and the GDP-fucose transporter (GDP-Fuc Tr). Our research group has previously reported an increase in α(1,6)-fucosyltransferase (FUT8) activity and expression in colorectal cancer (CRC) patients. Furthermore, we have demonstrated that FUT8 increased expression predicts patient's CRC recurrence. The aim of this study was to analyze the role of both, the FX enzyme and the GDP-Fuc Tr in the regulation of fucosylation in CRC. The FX enzyme is involved in the GDP-Fuc biosynthesis through de novo pathway, and GDP-Fuc Tr is responsible for the GDP-Fuc transport from the cytoplasm to the Golgi apparatus (where is a common donor substrate for fucosyltransferases). For this purpose, we assessed the immunohistochemical expression of FX and GDP-Fuc Tr in tumour and healthy specimens of colorectal tissue. FX expression was observed in 63.7% of tumoural tissue specimens (n = 91) and in 82.1% of their healthy counterparts (n = 28). The statistical analysis showed no differences in the enzyme expression between both tissues. GDP-Fuc Tr expression was detected in 84.3% and in 48.1% of colorectal tumour (n = 102) and healthy tissue (n = 27) specimens, respectively. The statistical comparison showed significative differences in the GDP-Fuc Tr expression between the colorectal tumour and the healthy tissue ($p < 0.001$, according to Wilcoxon test). On the other hand, the FX and GDP-Fuc Tr expression in colorectal tumour was correlated ($p < 0.001$, according to Wilcoxon test). Finally, to elucidate the prognostic value of FX and GDP-Fuc Tr in CRC, a survival analysis was performed. The results revealed that FX and GDP-Fuc Tr expression were not associated with the tumour recurrence. In conclusion, the GDP-Fuc Tr overexpression in CRC suggests that the GDP-fucose transport to the Golgi apparatus could be an important factor for increased fucosylation in this neoplasia.

P10-33
Protein engineering of lectins – in silico and *in vitro* approaches

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Protein-carbohydrate interactions are usually characterized by a low affinity for monovalent ligands that is balanced by multivalency resulting in high avidity for ligands with several potential epitops. However, recent characterization of lectins involved in pathogenesis has demonstrated their much higher affinity even towards monosaccharide than that observed for plant or animal lectins. This feature allows to use such lectins as a more/less specific tool, for example, for evaluation of protein glycosylation or isolation of glyco-conjugates. The contribution is focused on few examples where a combination of experimental methods (isothermal titration microcalorimetry, surface plasmon resonance, X-ray crystallography) with computational approaches can bring a significant profit for better understanding of protein-carbohydrate interactions on molecular level. Insight into structure correlation with function analysis of high-affinity lectins allows for construction of carbohydrate-binding proteins with tuned affinity and specificity for biotechnology and bioanalytical purposes.

P10-34
Protein trafficking in melanoma cells with altered glycosylation profile

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N-glycosylation is a modification of membrane and secreted proteins. It results from concerted action of glycosidases and glycosyltransferases in an endoplasmic reticulum and a Golgi apparatus. There are several examples for the importance of structural variety of glycans in protein sorting and cellular recognition (1). Due to the complexity and tissue-dependence, many aspects of these processes are still unknown. GnT-V (*N*-acetylglucosaminyltransferase-V) is a Golgi enzyme catalyzing a key step in biosynthetic pathway of N-glycan ligands for galectins (2). GnT-III (*N*-acetylglucosaminyltransferase III) is responsible for the formation of a characteristic structure known as 'bisected' N-glycan for the unusual conformation (3). We have studied localization of different proteins in culture of melanoma cell lines with altered glycosylation patterns due to stable overexpression of GnT-V (WM266-4-V) or GnT-III (WM266-4-III). Chosen proteins are involved in various processes like folding and quality control, vesicular transport, cell interactions, migration and signalling. All the cell lines have been previously obtained in our laboratory (4). Cell fractioning, Western blotting and confocal microscopy were used for the study. Subcellular localization of a protein may bring ideas for exploring its function and highlight the importance of *N*-glycosylation.

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P10-35
The human O-GlcNAcase C-terminal domain is a pseudo histone acetyl transferase

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The dynamic O-linked *N*-acetylglucosamine (O-GlcNAc) modification of specific serine and threonine on intracellular proteins in higher eukaryotes has been shown to directly regulate important processes such as the cell cycle, insulin sensitivity and gene transcription. Like protein phosphorylation, O-GlcNAc levels cycle in response to a wide variety of stimuli. The addition and removal of the GlcNAc sugar is catalyzed by O-GlcNAc transferase (OGT) and O-GlcNAc hydrolase (OGA) respectively. OGA is a large multi-domain enzyme and reported to be bi-functional, possessing glycosyl hydrolase and (histone) acetyl transferase (HAT) activity. Insights into the mechanism, protein substrate recognition/specificity of eukaryotic OGA (glycosyl hydrolase domain) have mainly been achieved via the use of close bacterial homologues. However, molecular details of the putative OGA

C-terminal HAT domain are largely unknown. Here we describe the crystal structure of a close homologue of the human OGA C-terminal HAT domain. Together with mutagenesis and biophysical data, the structure of the bacterial homologue reveals that the human OGA C-terminal HAT domain adopts a variant acetyltransferase fold. We show that, while the bacterial protein binds acetyl CoA, the eukaryotic OGA C-terminal HAT domain is unlikely to bind acetyl CoA, drawing into question the original report attributing HAT activity to this domain.

P11 – Membrane Transporters

P11-1
Channel-forming activity of cecropin A depends from membrane dipole modifiers in planar lipid bilayers

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Cecropins, positively charged antibacterial peptides found in the Cecropia moth, form the anion-selective channels of different conductance levels in planar lipid bilayers. We have studied the effect of membrane dipole modifiers (phloretin and RH 421) on the channel-forming activity of cecropin A in bilayers. Cecropin A was added to one-side of a membrane prepared from dioleylphosphoethanolamine (50 mol%) and dioleylphosphoserine (50 mol%) in 0.1 M KCl (pH 7.4) solution. The dipole modifiers were added to both-side of a membrane after the cecropin A. We found that addition of 20 μM phloretin to the bilayer bathing solution, known to decrease the membrane dipole potential, was accompanied by more than 15-fold decrease in the steady-state transmembrane current induced by cecropin A. At the same time, increasing the membrane dipole potential due to the introduction of 5 μM RH 421 led to more than 3-fold rise in the steady-state transmembrane current. Similar effects were observed in the presence of 33 mol% cholesterol in the membrane forming solution. The mechanism of the influence of phloretin and RH 421 on the channel-forming activity of cecropin A is discussed.

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P11-2
Molecular mechanisms of ABC transporters: insights from molecular dynamics simulations

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ABC transporters are membrane proteins that use the energy from ATP-hydrolysis to actively transport substrates across membranes [1]. All ABC transporters are composed by a minimum "functional core" of four modules: two transmembrane (TMDs) and two catalytic (NBDs) domains. Until this moment, and despite the large amount of experimental and theoretical data available, many fundamental questions about the ABC-family remain unanswered. In particular, it is still not clear which are the conformational changes induced by ATP-hydrolysis in the NBDs, nor how these rearrangements are "transmitted" to the TMDS.

The main objective of this work is to identify the structural changes occurring during an ATP-cycle in three distinct systems: an isolated NBD dimer from *Methanococcus jannaschii* [1], an ABC exporter from *Staphylococcus Aureaus* [2] and an ABC import system from *Escherichia coli* [3]. In this work, we present the results of three computational studies [1-3] using extensive Molecular Dynamics (MD) simulations of several intermediates states of the ATP-cycle. Our simulations allowed the identification of the hydrolysis-induced conformational rearrangements, not only in theNBDs, but also in the TMDs. Additionally, in the context of the complete transporters, we were able to identify, for the first time, the atomic details associated with the NBD dimer dissociation upon hydrolysis. Lastly, we suggest a general mechanism for coupling hydrolysis to substrate translocation, in which the NBDs "helical sub-domain" and the TMDs "coupling helices" are the keystones.

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P11-3
The role of interaction of mevastatin with different membrane components in reversing multidrug resistance of colon cancer cells

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Mevastatin is one of the most potent inhibitors of HMG-CoA reductase used for treatment of hypercholesterolemia. In the last few years statins have gained interest as cancer prevention agents. It was documented that the long-term use of statins reduced the risk of colorectal cancer and that these drugs might revert doxorubicin resistance in some cancer cell lines. In our studies we tested an ability of mevastatin to inhibit the growth of human adenocarcinoma cancer cells as well as the possibility to reduce drug resistance of LoVo/Dx cells by this statin. It was shown that mevastatin may act as antiproliferative agent because it inhibits the cell growth both in drug sensitive (LoVo) and drug resistant (LoVo/Dx) cell lines. The results of our studies indicate that mevastatin is able to reverse the resistance of colon cancer cells against doxorubicin and therefore this drug may be regarded in future as therapeutic agent improving doxorubicin efficacy during cancer chemotherapy. We also took an advantage of the fact that doxorubicin is highly fluorescent and can be used in confocal microscopy in order to analyze its accumulation inside the studied cells. The intensity of fluorescent signal in LoVo/Dx cells increased in the presence of mevastatin. The expression of three MDR-associated transporters (MDR1, MRP1, BCRP) in these cells has been studied by RT-PCR and immunohistochemical methods. Analysis revealed that in LoVo/Dx cells mevastatin increased the expression of BCRP. The ability of mevastatin to modulate multidrug resistance of cancer cells may be due in part to its interaction with lipid phase of membrane. The effect of mevastatin on biophysical properties of lipid membranes was studied by fluorescence spectroscopy and microcalorimetry techniques.

P11r-4
Overexpression or lack of AQP1 in the cell membrane modify cell proliferation associated to hypoxia

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Aquaporin-1 (AQP1) expression has been associated with tumor formation, particularly with angiogenesis, cell migration and proliferation. In the adult carotid body (CB), an oxygen-sensing organ that grows under chronic hypoxia, expression of AQP1 was detected in type I chemoreceptor cells and in endothelial cells

of small CB blood vessels. In the present work we explore the role of AQP1 in the cell proliferation process that occurs in the CB upon exposure to chronic hypoxia, comparing wild type animals for AQP1 expression (AQP1+/+) to nock out ones ($AQP1^{-/-}$). Also was analyzed how is affected the proliferation rate of PC12 cells with stable over expression of AQP1. Mice were either let in normoxia (21% of O_2), or maintained for 12 and 21 days in a hypoxic atmosphere (10% of O_2), and received BrdU treatment as a proliferation cell marker. Immunohistological analysis of the CB was used to quantify the number of TH+, BrdU+ and TH plus BrdU double-positive cells in normoxia and hypoxia conditions. Parenchyma CB total volume and TH+ cell volume were also measured. Lower number of total BrdU+ and TH-BrdU+ cells were observed in $AQP1^{-/-}$ mice after quantification at the two times of hypoxia indicated. By other hand, proliferation rate and cell cycle phases analysis performed in wild type PC12 cells and PC12 over expressing AQP1 showed that higher cell proliferation rate and larger percentage of cells in phase S and G2 were associated to AQP1 over expression. These results demonstrate a significant role of AQP1 in the cell proliferation process, but more studies will be necessary to understand the molecular basis that underline this phenomenon.

P11-5
The gold-based compound Auphen affects proliferation rate of AQP3 expressing cells

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The presence of AQP3 in the cell membrane has been correlated with higher entrance of glycerol to the cell, increment of ATP content and therefore larger proliferation capacity. Recently, the gold (III) complex Auphen has been described as a very selective and potent inhibitor of AQP3′s glycerol permeability. Here we explore the effect that this selective inhibitor produce on the cell proliferation of cell lines that considerable express AQP3 (A431) and compare it with cells with low/or none AQP3 expression (NIH-3T3 and PC12). Proliferation rate was evaluated by either counting the number of viable cells, with the trypan blue exclusion method using a Neubauer haemocytometer, after 24 and 48h of culture in the presence or not of 5mm Auphen; or by calculating after immunofluorescence analysis the percentage of cells that incorporate BrdU after 2, 4, 6, 8 and 24h of treatment, + or – Auphen. Cytotoxic effect of the compound was discarded after counting by flow cytometry a similar number of dead cells that incorporate propidium iodide (PI) in the absence or presence of twenty fold the concentration of Auphen used for the proliferation assays. Measurement by a colorimetric kit of cellular mitochondrial dehydrogenases activity, which is proportional to number of cells, also confirmed lack of drug's cytotoxicity. After ruling out a cytotoxic effect of Auphen, the reduction of number of cells observed in the A431 cell line after treatment with the compound confirmed a significant inhibitory effect over the cell proliferation process of cells that express AQP3. On the contrary, Auphen did not reduce the proliferation process in cells with not expression of AQP3. These results may indicate a potential therapeutic effect of Auphen over tumorogenis in tissues with large expression of AQP3 as the skin. Epidermoid carcinomas and other skin cancer types could be susceptible to Auphen treatment.

P11-6
Defining an export system for translation inhibitor Microcin C

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Microcin C (McC) is a modified heptapeptide that inhibits protein synthesis in sensitive bacterial cells. McC penetrates in cytoplasme through ABC trasporter Yej. Once in cytoplasm the peptide part of McC is processed by three aminopeptidases *E. coli* PepA, PepB or PepN. Processed McC is a potent inhibitor of aspartyl-tRNA sinthetase. Proteins responsible for McC synthesis, export and self-immunity of the producing cell are the products of the *mccABCDEF* gene cluster. The *mccA* gene codes for the McC heptapeptide. MccB, MccD and N-terminal domain of MccE are required for biosynthesis of McC. The C-terminal domain of MccE and MccF are responsible for self-immunity to McC. According to bioinformatics analysis MccC is a transmembrane protein that belongs to the MFS family transporters. This protein transports mature McC out of producing cells. Cells expressing *mccC* are also resistant to exogenous antibiotic. We have shown that cells overexpressing MccC are hypersensitive to kanamycin and synthetic analogs of processed McC. The reason of this hypersenstivity is still not known. We developed a procedure for purification of membrane fractions containing MccC and used it to identify *E. coli* proteins that interact with MccC to form an McC export system.

P11-7
Interaction of dipole modifiers with polyene-sterol complexes

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Polyene antibiotic amphotericin B (AmB) remains the drug of choice for treatment of patients with systemic fungal infections in spite of side effects. Biological activity of AmB is related to its ability to form pores in membranes of target cells. Recently we showed that effect of *dipole modifiers* (flavonoids and styryl dyes) on the conductance of single AmB channels resulted from changes of the membrane dipole potential. The present study examines the effect of dipole modifiers on the AmB multi-channel activity in planar lipid bilayers containing Chol or Erg. The addition of phloretin in Chol-containing membranes leads to increase of the steady-state AmB-induced transmembrane current (*I*). Quercetin significantly decreases and RH421 increases *I* in Erg-containing bilayers. In other cases, the introduction of dipole modifiers does not change *I*. The obtained effects may rather be attributed to direct interaction of dipole modifiers with AmB/sterol complexes and not to effect of the dipole potential. The formation of AmB/Chol-complex prevents sterol from close contact and formation of H-bond with AmB polar head in contrast to AmB/Erg-complex (Neumann *et al.,* 2010). We speculate that phloretin plays a role of a mediator for the formation of hydrogen bond network between Chol and AmB which stabilizes the complex due to formation of additional point-to-point interactions maximized van der Waals forces. RH421 may participate in π-π electronic interactions in rigid AmB/Erg complex due to its polyene fragment and may also inset its polar groups to hydrogen bond network. Inclusion of quercetin in AmB/Erg-complex leads to its destabilization.

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P11-8
Gestational diabetes reduces placental uptake of arachidonic and docosahexaenoic acids and interferes with trophoblast development

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The long-chain polyunsaturated fatty acids (LC-PUFAs) arachidonic (AA) and docosahexaenoic (DHA) acids are essential for fetal development. Gestational diabetes mellitus (GDM) is a pregnancy disorder associated with perinatal complications and lifelong risk of metabolic diseases for the offspring. Our aims were: (i) to investigate whether uptake of 14C-AA and 14C-DHA is changed in human trophoblasts from GDM-affected pregnancies (DTB cells), when compared to uptake in human trophoblasts from normal pregnancies (NTB cells), and in response to specific GDM-associated conditions, and (ii) to compare viability, proliferation, differentiation and apoptosis in NTB and DTB cells. Uptake of 14C-AA and 14C-DHA by NTB cells was: (i) mediated by a saturable (protein-mediated) and a non-saturable (passive diffusion) mechanism; (ii) acidic pH-stimulated; (iii) inhibited by long-chain fatty acids; (iv) inhibited (14C-AA) or stimulated (14C-DHA) by phloretin, stimulated by DIDS, and not inhibited by ATP depletion; and (v) dependent on long-chain acyl-CoA synthetase (ACSL) activity. DTB cells showed a markedly lower capacity for 14C-AA and 14C-DHA accumulation over-time, through a decrease in the saturable and non-saturable components of uptake, which was associated with a decrease in ACSL1 mRNA levels. In addition, uptake of LC-PUFAs in NTB cells was increased after short-term exposure to TNFα (14C-AA and 14C-DHA) or insulin (14C-DHA). Finally, DTB cells showed higher proliferation and apoptosis rates than NTB cells. In conclusion, placental transport of LC-PUFAs is decreased in GDM, which probably contributes to the deleterious effects of this disease for the fetus.

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P11-9
Regulation of amino acid transporter $ATB^{0,+}$ by protein kinase C

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$ATB^{0,+}$ (*SLC6A14*) is an amino acid transporter, capable of transporting a broad spectrum of neutral and basic amino acids with a highest affinity towards isoleucine, leucine and phenylalanine and a low affinity for carnitine. Its expression has been observed to be augmented in malicious cell lines. We cloned and heterologously expressed the rat gene coding 3xFLAG-tagged $ATB^{0,+}$ in HEK 293 cells and found activation of L-leucine transport by protein kinase C (PKC) activator - phorbol-12-myristate-13-acetate and inhibition by a general (bis-indolylmaleimide II) and classical isoforms (Gö 6976) PKC inhibitors. Transport stimulation was correlated with phosphorylation of $ATB^{0,+}$ on serine moiety and augmented plasma membrane presence of the transporter, as verified by biotinylation studies with membrane non-permeable reagent, EZ-Link® Sulfo-NHS-LC-Biotin. Immunoprecipitation experiments with anti-FLAG affinity gel demonstrated $ATB^{0,+}$ interaction with PKCα, but not with other classical or novel PKC isoforms. The observed regulation of $ATB^{0,+}$ by PKC correlates with a high activity of both proteins reported for cancer cells and confirms an important role of $ATB^{0,+}$ in delivering necessary amino acids for quickly proliferating cells.

P11-10
Fluorone dyes interactions with Na,K-ATPase and its large cytoplasmic loop

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Fluorone dyes are hydroxy-xanthene dyes. The xanthene dyes are among the oldest synthetic dyes and have many important applications. Xanthene (fluorone) dyes are often used as a drug components (virostatic drugs), laser dyes, in food industry for food colouring, as photosensitizers in photodynamic therapy of cancer or in medicine as a tool for diagnostic. These compounds, which include fluorescein, rose bengal, eosin Y and erythrosine, have almost the same ring skeleton. Na,K-ATPase is possible target for all of these described dyes. Moreover, Na,K-ATPase belongs to the family of membrane proteins, which are in general extremely potent drug targets due to their role as transporters in the interaction between cells and cellular compartments. This role together with the fact, that Na,K-ATPase has highly exposed position in the cell, renders it of most importance in cellular physiology. In this work the combination of steady-state and time-resolved fluorescence methods together with molecular docking were applicated to observe interactions of fluorone dyes with Na,K-ATPase. In view of the fact that dyes should occur in cytoplasm, we expect that they will interact with cytoplasm part of the enzyme. Because of this presumption, also interaction with large cytoplasmic loop of Na, K-ATPase (C45) was tested. We were able to identified two binding sites. First is located on the transmembrane part of the protein. Second binding site is located on the large cytoplasmic loop of Na,K-ATPase (C45) alpha subunit.

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P11r-11
New insights into the functional characteristic of GLUT12: Na^+/Glucose cotransport and glucose induced outward chloride currents

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GLUT12 is one of the last facilitative glucose transporters cloned. During the last years, research has focused on the study of GLUT12 as a secondary insulin sensitive transporter, and its role on impaired insulin-signaling pathologies. Its expression in some tumor tissues has been described and recently, it has been proposed as one of the key proteins in the glucose supply to malignant cells. Functional studies have showed that GLUT12 transports sugars down its concentration gradient and also that it can work as proton-coupled symporter. Although glucose has been described as its main substrate, GLUT12 can transport other sugars as fructose or galactose. Previous studies from our laboratory, performed in *Xenopus laevis* oocytes expressing GLUT12, showed that glucose uptake increased in the presence

of Na^+ and induced ionic currents. In the present work, using $^{22}Na^+$, we demonstrate that Na^+ can entry into the cell through GLUT12, and that its entry increases in the presence of glucose, suggesting that GLUT12 could act as a Na^+/Glucose cotransporter. Moreover, studies using electrophysiological techniques showed that glucose induced currents are elicited by activation of a chloride channel. Those currents were inhibited by some classic chloride channel inhibitors (niflumic acid, NPPB and DPC), and showed anion selectivity (NO_3 > I > Br > Gluconate) different for that described for *Xenopus laevis* endogenous chloride channel, indicating that this activity was linked to GLUT12. Furthermore, glucose activated outward chloride currents were uncoupled to glucose uptake, as demonstrate by the fact that glucose transport was not inhibited by niflumic acid, suggesting that current activation could be due to substrate binding and not to glucose transport.

In summary, GLUT12 can couple glucose transport to Na^+ and glucose induces outward chloride currents, which could be activated by substrate binding to the transporter.

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P11-12
Functional investigation of MexB, a multidrug transporter from *Pseudomonas aeruginosa*

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Among the various mechanisms developed by the bacteria to counter to the effect of antibiotics, active efflux is on the front line. In Gram-negative bacteria, efflux transporters are organized as multicomponent systems where the efflux pump, located in the inner membrane, works in conjunction with a periplasmic protein and an outer membrane protein. The cytoplasmic inner membrane protein acts as an energy (pmf)-dependent pump with broad substrate specificity.

We describe an original activity assay for membrane transport that uses the proton motive force-dependent efflux pump MexAB from *Pseudomonas aeruginosa*. This pump is co-reconstituted into proteoliposomes together with bacteriorhodopsin (BR), a light-activated proton pump. In this system, upon illumination with visible light, the photo-induced proton gradient created by the BR is shown to be coupled to the active transport of substrates through the pump.

P11-13
Unravelling supramolecular organisation of membrane proteins by solid state NMR and Molecular Dynamics simulations

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Biological functions are critically modulated by the dynamic interplay between membrane-bound proteins and their membrane environment. Such supramolecular arrangements are hitherto scarcely understood on a molecular basis and their determination poses a great challenge for structural biology.

While solid state Nuclear Magnetic Resonance (ssNMR) principally allows probing protein supramolecular structure in native-like membranes, the interpretation and completion of these data remain formidable quests, rendering supramolecular structures of membrane proteins at atomic resolution experimentally almost elusive. Here we introduce an approach that combines ssNMR spectroscopy and Molecular Dynamics (MD) simulations for the determination of the supramolecular structure of membrane-bound proteins with a resolution and level of accuracy difficult to obtain by either method alone. Our ssNMR-MD joint-approach is demonstrated on the membrane-associated Shaker B peptide that is representative for N-type inactivating domains of voltage gated K^+ channels [1].

In an extensive study of K^+ channels KcsA and KcsA-Kv1.3, we show by correlating ssNMR experiments and MD simulations that the membrane environment induces considerable structural differences at the membrane/protein interface in comparison to protein crystal and micelle structures, which is presumably important for channel gating, and we investigate the different lipid specificities of both K^+ channels. These studies highlight the stunning influence of the protein – membrane dialogue on membrane protein structure and function.

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P11r-14
The expression of sodium-dependent vitamin C Transporters SVCT1 and SVCT2 in liver cells is affected by oxidative stress

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In humans, the bioavailability of vitamin C mainly depends on its uptake by vitamin C transporters, SVCT1 and SVCT2. The aim of this study was to investigate the effect of several diseases accompanied by metabolic and oxidative stress on the expression of SVCT1 and SVCT2 in liver and extrahepatic tissues involved in ascorbic acid homeostasis. Using real-time RT-PCR, up-regulation of SVCTs in the liver of patients with cirrhosis, haemochromatosis, cholestasis and non-alcoholic steatohepatitis was found. Obstructive cholestasis in the rat induced up-regulation of Svct2 and down-regulation of Svct1 in the liver, but a reduction in the expression of both transporters in ileum, whereas no change in kidney, brain, and lung was detected. In obese Zucker rats, the levels of mRNA for both Svcts were similar to those found in lean rats, except for lung tissue, where Svct2 was up-regulated. In streptozotocin-induced diabetic rats down-regulation of Svct1 in liver and kidney and down-regulation of Svct2 in kidney and brain was observed. Because all experimental models used here shared as characteristic the appearance of hipercholanemia, the effect of bile acids exposure was investigated using rat hepatoma Can-10 cells. Incubation with taurocholic acid or taurochenodeoxycholic acid, but not tauroursodeoxycholic acid resulted in up-regulation of both Svcts. However, as demonstrated in human hepatoma Alexander cells transiently transfected with FXR/RXRα, this effect was not dependent on the nuclear receptor FXR. In sum, metabolic and oxidative stress induce different responses regarding the expression of Svct1 and Svct2 in the liver and other organs, which may play an important role in the ability of cells to take up vitamin C under physiological and pathological conditions.

P11-15
Aquaporin inhibition by a gold-based compound

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Aquaporins (AQPs) are membrane channels that conduct water and other small solutes. They can be divided into two major groups: those strictly selective for water (orthodox aquaporins) and those that also conduct small solutes including glycerol (aquaglyceroporins). Aquaporins serve in many physiological functions and are involved in numerous pathophysiological conditions such as kidney diseases, cancer, obesity, glaucoma, brain edema and epilepsy; therefore, aquaporin-based modulator drugs are predicted to be of broad potential utility in the treatment of several diseases. Despite some compounds have been described as aquaporin inhibitors, few are considered as suitable candidates for clinical development and none of them showed specificity for aquagliceroporins. Using screening system based on a stopped-flow permeability analysis of human red blood cells, we found a potent inhibition of the AQP3 aquaglyceroporin by gold(III) complexes. This effect was further confirmed using AQP3-transfected PC12 cells. Among the various metal compounds tested, Auphen was the most active on AQP3 poorly affecting the water permeability of the orthodox aquaporin AQP1. The mechanism of gold inhibition is related to the ability of Au(III) to interact with sulphydryls groups of proteins such as the thiolates of cysteine residues. Additional docking studies with a human AQP3 homology model evidenced the thiol group of Cys40 as a likely candidate for binding to gold(III) complexes. The high selectivity and low concentration dependent inhibitory effect of Auphen (in the nanomolar range) together with its high water solubility makes the compound a suitable drug lead for future *in vivo* studies. These results may present novel metal-based scaffolds for AQP drug development.

P11r-16
Enhancing the expression of the drug transporter OATP1B1 as a potential strategy to overcome chemoresistance in liver tumours

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In liver tumours, the efficacy of anticancer chemotherapy is often very poor due to the efficient export of the active agents, which is mediated by ATP-binding cassette (ABC) proteins, such as MRP2. To counterbalance this mechanism of chemoresistance, we have investigated the usefulness of inducing in tumour cells the expression of a multidrug uptake transporter OATP1B1 under the control of MRP2 promoter (MRP2pr). Either full-length or partially deleted sequences of MRP2pr were cloned into plasmids containing the ORF of firefly luciferase. The constructs were transfected into human hepatoma cells (PLC/PRF/5). The MRP2pr activity was stimulated with dexamethasone and cisplatin and the fragment able to induce the highest response (−873 to +1 bp in MRP2pr) was cloned upstream of the OATP1B1 ORF. As determined by RT-QPCR and Western blot, transfection of hepatoma cells with this chimera induced a 20-fold increase in OATP1B1 expression. Exposure to dexamethasone further increased OATP1B1 expression. V5-tagged OATP1B1 was immunolocalized at the plasma membrane of hepatoma cells. Transport studies indicated that the protein was functional. Indeed MRP2pr-OATP1B1-transfected cells were more sensitive to antitumor OATP1B1 substrates, such as paclitaxel, methotrexate and the cisplatin-bile acid conjugates Bamet-R2 and Bamet-UD2. MRP2pr-OATP1B1-induced enhanced drug sensitivity was further increased (x2) by treatment with dexamethasone. In conclusion, transfection of tumour cells with chimeric DNA containing the ORF of an uptake transporter under the control of the promoter of a resistance-associated protein may constitute an interesting strategy to overcome the chemoresistance characteristic of hepatocellular tumours.

P11r-17
Biogenesis and assembly of EmrE, a dual topology membrane protein

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EmrE is a small multidrug resistance protein from *Escherichia coli* that extrudes toxic compounds across the plasma membrane in exchange for protons, conferring resistance to drugs such as ethidium bromide, methyl viologen or acriflavine. It is a dual-topology membrane protein, meaning that it is able to insert into the membrane in two opposite orientations. We showed that the orientation of the protein can be manipulated by the addition of a single positively charged residue in the parts of the protein that flank the four transmembrane helices, regardless of position. Two EmrE variants with fixed orientation were engineered by adding positively charged residues in the loops, EmrE(Cin) with its N- and C-terminus facing the cytoplasm, and EmrE(Cout), with its N- and C-terminus in the periplasm. The functional unit of EmrE is a homodimer. Using an *in vivo* assay it was shown that only when EmrE(Cin) and EmrE(Cout) are co-expressed and able to form antiparallel dimers, they are active. However, there is some controversy around the relative orientation of the monomers in the dimer. Using BN-PAGE and cysteine disulfide bond formation we have recently been able to show that although parallel dimers can be formed, antiparallel dimers are preferred. We conclude that the antiparallel dimer is not only the more active, but also the more stable form.

P11-18
Thyroid hormone action in the brain of mice with inactivated *Slc16a2* and *Slc7a8* genes

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Thyroid hormone (T4 and T3) entry into cells is facilitated by transporters of several transmembrane protein families. The monocarboxylate transporter (MCT) 8 is specific for T4 and T3 transport. Mutations of the *SLC16A2* gene, encoding MCT8 cause an X-linked syndrome of profound neurological impairment and altered metabolism and circulating concentrations of thyroid hormones. The syndrome is due to the failure of thyroid hormone to reach the neural target cells in adequate amounts to sustain normal brain development. The syndrome is partially reproduced in mice with inactivated *Slc16a2* gene. These Mct8-deficient mice show the same alterations of thyroid hormone concentrations as the patients, but no signs of neurological impairment. It is possible that the reason for the phenotypic discrepancy between the knock out mice and the patients is due to the presence in mice of alternative transporters that may compensate for the lack of Mct8. Among possible candidates we have analyzed the role of the heterodimeric aminoacid transporter Lat2 (Slc7a8). We have generated mice with inactivated *Slc16a2* and *Slc7a8* genes and analyzed the expression of thyroid hormone-dependent genes in the cerebral cortex of developing (postnatal day 21) and adult mice. The results show that single inactivation of the *Slc7a8* gene does not interfere with thyroid hormone action in brain. Furthermore thyroid hormone-dependent genes show similar patterns of expression in the single *Slc16a2* knock out mice than in the double *Slc16a2* and *Slc7a8* knock outs. We conclude that it is unlikely that compensation by Lat2 is the reason for the absence of neurological impairment in the Mct8-deficient mice.

P11r-19
Effect of acetaminophen treatment on the role of export pumps ABCB1, ABCC1-4 and ABCG2 in the placental barrier

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Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. Toxic doses of APAP induce upregulation of ABC transporters in liver cells, whereas subtoxic doses induce upregulation of ABCB1 in both liver and intestine. The aim of this study was to evaluate the placental expression of ABC proteins in response to non-toxic doses of APAP. In pregnant rats, the administration of APAP over the last three days of pregnancy resulted in an enhanced levels of Abcb1, Abcc1-4 and Abcg2 mRNA in intestine, and kidney. In placenta, Abcb1, Abcc1 and Abcg2 mRNA were also increased but, interestingly, the protein levels of Abcg2 were decreased. To investigate the effect of APAP on the rat placental barrier, complete obstruction of the maternal common bile duct was imposed on day 19 of gestation. This resulted in enhanced levels of bile acids in the maternal serum, but also in placenta and foetal liver, kidney and serum. The effect was more marked if cholestatic mothers were treated with APAP. Incubation of human cells from hepatoblastoma (HepG2), hepatocarcinoma (SK-HEP-1), colorectal adenocarcinoma (LS 174T and Caco-2), kidney (HK-2), and choriocarcinoma (BeWo, Jeg-3, and JAr) with subtoxic doses of APAP resulted in tissue-specific changes regarding the expression of ABC transporters. Thus, in HepG2, LS 174T, and Caco-2 cells, ABCB1 expression and the ability to export rhodamine 123 were enhanced. In HK-2 cells, ABCB1 was upregulated but this was not accompanied by enhanced export activity. The expression of ABCC1 was not modified in any cell type, whereas these of ABCC2, ABCC3, and ABCC4 were significantly decreased in SK-HEP-1 and HK-2 cells. The ability of ABCC1-4 to export calcein from HK-2 cells was also reduced. The expression of ABCG2 together with its ability to export bodipy-prazosin were reduced only in Jeg-3 cells. These results suggest that APAP induces a tissue-specific effect. During pregnancy the treatment with this drug may alter the efficacy of the placental barrier, which may reduce the protection of the foetus against potentially toxic Abcg2-substrates present in maternal blood.

P11-20
Copper (I) ATPases – a correlation between topology and transport mechanism?

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The type IB subfamily (P_{IB}) of P-type ATPases is present in all kingdoms of life and responsible for the active transport of heavy metal ions across biological membranes. As such, they are crucial for heavy metal homeostasis and detoxification by the ATP-driven extrusion of heavy metals from the cytoplasm of cells. Recently, the first crystal structure of a P_{IB}-type ATPase, the Cu(I) ATPase CopA from *Legionella pneumophilia* (LpCopA) in a copper-free E2-P_i transition state of dephoshoprylation was determined at 3.2 Å resolution [1].

Now, we have determined a structure of LpCopA representing a the fully phosphorylated copper-free (E2P) state at 2.75 Å resolution (Mattle et al, unpublished). Together, the structures reveal distinct structural characteristics of the subgroup such as a large amphipathic helix at the cytoplasmic interface, and they suggest several, transient binding sites along a putative copper transport pathway across the membrane. Unexpectedly, the two structures are however relatively similar in the transmembrane region compared to the equivalent structures of the sarcoplasmic/endoplasmatic reticulum Ca2+-ATPase (SERCA). These findings suggest that the conformational changes associated with dephosphorylation of CopA may be different to those of SERCA. We discuss these observations with respect to functional and topological differences as well as MD simulations.

References

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P11r-21
Subcellular localization of potassium and sodium in wild-type and vacuolar mutants of *Saccharomyces cerevisiae*

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The level of intracellular ions in a living cell is assumed to be controlled by the plasmalemma as well as by the organelle transport systems. The participation of organelles in the regulation of ion concentrations in the cytosol could result in the compartmentation of ions. Potassium is a fundamental cation for *Saccharomyces cerevisiae*; it stimulates fermentation and respiration and it participates in the modulation of the internal pH. *Saccharomyces cerevisiae* cells contain a concentration of potassium of around 200–600 nmols of K^+/mg of cell and although it is usually accepted the importance of its intracellular distribution in the performance of the cell, paradoxically it has never been definitively established a reliable protocol to study this process. In fact most of the few studies trying to determine subcellular cation localization in yeasts have focused on vacuole and cytoplasm, which turned out to be a too simplistic approach.

By using several specific procedures, we were able to isolate vacuoles, mitochondria and nuclei. We determined the purity and efficiency of our isolation procedures and we were able to measure K^+ and Na^+ levels in the mentioned organelles and to deduce the cytoplasmic content in several yeast strains and under different growth conditions. Our results show differences in intracellular distribution of K^+ and Na^+ between several compartments of the cell and indicate a tight regulation of the cytoplasmic potassium content when the cation is scarce in the medium.

P11-22
Functional characterization of K transporters in *Synechocystis* sp. PCC 6803

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In all living cells, K is the major cation used for the maintenance of turgor pressure, cytosolic osmolality and membrane potential. The genetic and biochemical studies indicate that bacteria react to hyperosmotic stress, and that K uptake and K extrusion play an important role in the regulation of cell volume and the maintenance of the ionic balance within the cell. *Synechocystis* sp. 6803 contains two genes encoding the Ktr-type K transporter and the possible Kdp-type K transporter. In our previous studies have shown that *Synechocystis* Ktr is essential for the adaptation of the cell to osmotic stress. It has been shown that in growth media that contain low concentrations of K, Kdp functions as a K uptake system in *E. coli*. However, additional physiological roles of Kdp remain to be elucidated and not much is known about the Kdp transporter from *Synechocystis*.

In order to elucidate the role of the *Synechocystis* Kdp transporter, the *kdp operon* was heterologously expressed in the K-uptake deficient *E. coli* strain LB2003. The LB2003 cells expressing Kdp showed significant high K uptake activity, compared with the empty vector control. However K uptake by Kdp-expressing cells was lower than that of cells expressing Ktr. To examine whether Ktr and Kdp may be involved in the adaptation to hyperosmotic stress in *Synechocystis*, osmotic shock assay was performed using the mutants of the two K-transport systems. The treatment for 3 M sorbitol for 4 h strongly decreased the growth rates of both the *ktr* deletion strain and the *ktr*, *kdp* double deletion strain while the wild type grew well. The *kdp* deletion strain was also decreased to less extent. In *Synechocystis* the Kdp system contributed to the uptake of K into the cell after hyperosmotic shock, which suggests that the Kdp system cooperates with the Ktr system to adapt to the sudden change in the osmotic strength of the surrounding medium in the initial cellular response.

P11-23
Studies of multidrug efflux pump activity in *Lactococcus lactis* using ethidium cations

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Multidrug resistance (MDR) efflux pump is the main reason of bacteria resistance to antibiotics. *Lactococcus lactis* is very important bacterium in food industry. We used spectrofluorimetric measurments to assay the activity of MDR pumps and to investigate the response of the pumps to cell growth conditions. We used ethidium (Et) as as indicatory compound, which increases its fluorescence because of the binding to DNA. We also used another MDR pump substrate tetraphenylphosphonium (TPP^+) as a competitive compound and antibiotic gramicidin D as the cell permeabilizer. In this study we demonstrated that the differences in MDR efflux pump activity depend on the growth conditions (with or without heme source) and the energetic state of cells.

P11-24
Vitamin C transporters in sertoli cells and their relevance to the blood-testis barrier in the protection against oxidative stress

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In the testis, germ cells are isolated from the systemic circulation by the blood-testis barrier, which consists of a basal layer of Sertoli cells associated through an extensive array of tight junction complexes. Vitamin C and glutathione are potent cellular antioxidants, essential for the development of germ cells. To study the behaviour of Sertoli cells as a first approach towards the molecular and functional characterization of the vitamin C transporters and its relationship with different concentrations of glutathione, we used the murine 42GPA9 immortalized Sertoli cell line. RT-PCR analyses revealed that these cells express both vitamin C transport systems, a finding that was confirmed by immunocytochemical and immunoblotting analysis. The kinetic assays using radioactive ascorbic acid revealed that both, ascorbic acid transporters (SVCTs) and facilitative hexose transporters (GLUTs) are functionally active in these cells. Also, we demonstrated that Sertoli cells are capable to form a sealed monolayer and to incorporate ascorbic acid from both apical and basolateral surfaces. When Sertoli cells were treated with L-Buthionine-Sulfoximine, inhibitor of gamma-glutamylcysteine synthetase, we demonstrated that 42GPA9 Sertoli cells presented lower expression of SVCT1, while SVCT2 remained unchanged. Similar results were obtained when rats were treated with BSO and testis sections were ana-

lyzed. Altogether these results indicate that active vitamin C transporters (SVCTs) are expressed in Sertoli cells and that there is a close relationship between intracellular concentrations of glutathione and SVCTs expression. (FONDECYT 1060135, 1110508 IIC, DID UACh S-2010-29, Dirección de Investigación y Desarrollo, Universidad Austral de Chile).

P11-25
Protein tau – mediated effects on rat hippocampal transporters CHT1 and tau - amyloid beta interactions

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It is suggested that intracellular tau protein, when released extracelularly, could evoke direct toxic effects on cholinergic neurotransmitter system e.g. through muscarinic receptors and thus contribute to the pathogenesis of Alzheimer disease. In this study, we evaluated *in vitro* effects of six naturally occurring tau isoforms on rat hippocampal synaptosomal choline transporters CHT1 (large transmembrane protein associated with a high-affinity choline transport and vulnerable to actions of amyloid beta peptides *in vitro* or *in vivo*). Tau isoforms at nM concentrations inhibited the choline transport in a dose- and time-dependent saturable manner (352 = 441 > 410 = 383 > 381 = 412) and changes were associated rather with those in Michaelis constant than in maximal velocity. Moreover, actions of tau 352/441 were not influenced by previous depolarization of synaptosomes or by previous depletion of membrane cholesterol. Specific binding of [3H]hemicholinium-3 was not significantly altered by tau 352/441 at higher nM concentrations. It seems therefore that protein tau, similarly as amyloid beta peptides, can contribute to the pathogenesis of Alzheimer disease through transporters CHT1, however, interaction mechanisms are quite different (tau exerts its effects probably through direct interactions with extracellular domains of CHT1 without influencing choline recognition site, amyloid beta rather through lipid rafts in surrounding membrane). Results of our *in vitro* test performed on CHT1 transporters from cholesterol-depleted synaptosomes revealed interactions of tau 352 (but not of tau 441) and of amyloid beta 1–40. The interaction of tau and amyloid beta was evaluated also via surface plasmon resonance biosensor.

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P11-26
Implications of altered glucose transport observed in glucose transporter deficiency disease (GLUT1DS) on transport models

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Reduced glucose transport across the blood-brain barrier due to mutated forms of the glucose transporter GLUT1 (SCLA-1) results in Glut-1 deficiency syndrome (Glut-1DS, OMIM 606777), characterized by infantile seizures, developmental delay, acquired microcephaly, spasticity, ataxia, low cerebrospinal fluid glucose concentration, termed hypo-glycorrhachia and defective glucose transport in the patients' erythrocytes.

The missense glucose transporter mutant T295M, present in the exofacial link between transmembrane helices 6 and 7 causes Glut-1DS, but unusually, at 4°C *zero-trans* net influx of 3-*O*-methyl D-glucose (3OMG) or 2-deoxy-D-Glucose (2-DG) into the patients' erythrocytes is unaffected. However, at 37°C, net influx V_{max} of 3OMG and 2-DG into oocytes expressing T295M GLUT1 mutant are reduced by 30% and 50% respectively; whereas K_m and V_{max} of net 3-OMG efflux are reduced by 80%.

In-silico docking studies with Autodock Vina confirm the coexistence of multiple hexose docking sites in GLUT1, one closely adjacent to T295 in the external vestibule of the transporter. Several methionine rotamers at M295 can occlude hexose docking to this site.

A multisite kinetic model can simulate both the asymmetric reductions of V_{max} and K_m of 3OMG *zero-trans* influx and efflux at 37°C and the absence of inhibition of influx at 4°C similar to those previously described. None of these effects are explicable with the conventional alternating access model of glucose transport.

These studies also explain how low temperature transport assays with patients' erythrocytes may overlook some GLUT1DS phenotypes.

P11-27
Investigated freezing methods from water dynamics by aquaporin

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The frozen food and cells are widely used in our life. There are many points of freeze technology which for maintaining delicacy, such as improvement in the probability of survival of a cell, food, and a fresh fish, itself is developing, and should be improved. Especially the drip phenomenon at the time of dissolution causes degradation of the taste and quality. This phenomenon is caused the cell membrane damage.

It is the osmotic pressure difference of the inside and outside cell (water permeability) and makes a formation of ice nuclear by dramatic water structure change (water dynamics). In this research, water dynamics paying attention to the role of the aquaporin (AQP). One of AQPs family: AQP3 participated in osmotic pressure mitigation and ice nuclear prevention on the slow freezing (Ref. Biology of Reproduction. 121–124, 2007). Participation of AQP on the quick freezing with be used widely, but it is still unknown. This research evaluates what kind of influence of water permeability and the water dynamics by AQP on the quick freezing.

At first, we are established the stable cell lines introduced the AQP1, 4 or not. These cell lines are compering the cell viability and growth speed on the each condition cells. These results will be displayed at a conference.

P11-28
Towards the atomic structure of heteromeric amino acid transporters

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its, the heavy (rBAT or 4F2hc) and the light (7 transporters in humans). The light subunit is the catalytic one. The structural information of HATs is scarce. The heavy subunits are type II membrane N-glycoproteins with a large extracellular domain, which has sequence and structural homology to bacterial glucosidases. The light subunits have 12 transmembrane domains. The present structural paradigm of the human light subunits, AdiC, the bacterial arginine/agmatine exchanger, has the LeuT-fold, which is characteristic of several transporter families. The point mutations N22A and N101A in AdiC were crucial to unveil the crystal structures of this transporter in the outward-facing conformations with bound L-arginine, and in the open and occluded states, respectively. These structures helped to unravel the mechanisms of substrate binding and substrate induced-fitting mechanisms of AdiC. Unfortunately, the low amino acid sequence identity (17–19% amino acid sequence identity to human light subunits) precludes the generation of robust structural models for the light subunits of HATs, and obviously offers no clues on the macromolecular organization of the metazoan holotransporters. To approach the atomic structure of HATs, light subunits of HATs of different origins (from fly to human) were cloned and expressed in yeast. This screening resulted in a light subunit with a melting temperature (T_M) >40°C and a heterodimer with a T_M >65°C. Negative stain transmission electron microscopy and single particle analysis of this 4F2hc heterodimer was used for 3D reconstruction yielding the first structural model of a HAT at 19 Å resolution.

P11-29
Cholesterol uptake in Mycobacterium smegmatis

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Cholesterol uptake in Mycobacteria has been associated to the expression of the *mce4* operon, one of the *mce* loci found in Actinobacteria that are involved in different transport functions (e.g., lipid transport, cholesterol) and that also behave as virulence factors in *M. tuberculosis*. The *mce* operons contain 8–10 genes that encode two transmembrane proteins homologous to the permease sub-units of ABC transporters, and several putative cell-surface proteins of unknown function. Since Mce are ATP-driven transport systems it has been proposed that a single ATPase, not encoded in the *mce* operons, provides the energy required for substrate uptake. In *M. smegmatis*, that contains six *mce* operons, we have proposed that the *MSMEG_1366* gene encoding an ATPase is the one strictly required to provide the energy for cholesterol uptake by the Mce4 system. In order to study the role and the regulation of the *MSMEG_1366* gene, we have investigated its expression by RT-PCR observing that this gene is expressed at higher levels in cells growing in cholesterol as the only carbon and energy source, when compared with the basal levels observed in glycerol containing media. Remarkably, a constructed ATPase deletion mutant was unable to grow in cholesterol, and the capacity of using this carbon source was restored by complementation of the disrupted mutant, reinforcing our hypothesis about its critical role in cholesterol uptake. These results have been confirmed by radioactive cholesterol uptake assays. Interestingly, the ATPase mutant forms clumps when growing in liquid cultures and shows significant alterations in colony morphology in solid media, suggesting that the ATPase is probably involved in other physiological phenomena through its interaction with the other Mce transporters. Given the importance of this ATPase in cholesterol active transport and its possible function in other uptake or membrane processes, the protein has been cloned and overexpressed in *E. coli*, to facilitate its purification and crystallization in order to elucidate its structural and functional properties.

P11-30
Finding each other in the membrane: shall we meet in the genome?

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Escherichia coli as a model system, which exports cysteine and glutathione. The *cydD* and *cydC* genes are contiguous, the start codon of *cydC* follows the stop codon of *cydD*. We have created a *cydDC* knock-out strain and developed a growth assay that allows us to assess and quantify reconstitution of a functional transporter. Further, we can monitor dimer formation by Blue Native PAGE.Our results indicate that proteins synthesized from one mRNA are not committed to each other but can freely pair in the membrane. However, high subunit concentrations are needed for this to happen; at low expression levels, interactions between subunits originating from the same operon are preferred. These observations point to a scenario of concerted folding and/or reciprocal stabilization of the CydD and CydC subunits and suggest that an operon arrangement facilitates complex formation at low expression levels. Whether these observations are applicable to other membrane protein complexes remains to be studied.

P11-31
Embryonic chicken and murine chondrogenic cells express TRPV1 channels that influence cartilage formation

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Proper skeletogenesis requires ability of differentiating cells to detect environmental changes. Ion transport mechanisms through plasma membrane during chondrogenesis are poorly explored, but the essential role of characteristic intracellular Ca^{2+}-concentration changes in chondrogenic cells has been reported by our laboratory. The polymodal Transient Receptor Potential Vanilloid (TRPV) subfamily of non-selective cation channels is a good candidate to translate various extracellular stimuli into intracellular ion-concentration changes.

Our experimental model is a primary chondrogenic high density cell culture system (HDC); cells are isolated from limb buds of 4-day-old and 11.5-day-old chicken and murine embryos, respectively. Spontaneous cartilage formation occurs within 6 days in HDC.

Cells of HDC expressed mRNAs of various TRPV members during the course of cartilage differentiation; nonetheless, we focused on TRPV1 characterized by high Ca^{2+} permeability. Expression of TRPV1 mRNA and proteins showed good correlation to the differential status of chondrogenic cells. Avian TRPV1 is sensitive to heat stimulus and low pH but insensitive to vanilloid-like pharmacological modulators. Therefore, we applied heat treatment on chicken HDC and vanilloid-like

pharmacons on murine HDC. Both treatments increased the amount of metachromatically stained ECM. Intracellular calcium concentration was elevated by heat stimuli in chicken cells, indicating the presence of thermosensitive channels. This rise of Ca^{2+}-concentration did not require extracellular Ca^{2+}. Investigation of TRPV1 on murine chondrogenic cells is in progress.

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P11-32
Lipids role in COPII vesicle formation

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In the cell, transport of cargoes between organelles is mediated mainly by vesicles. This vesicles are formed thanks to proteins which polymerize as coats at membrane surfaces. Coated Protein complex II (COPII) is involved in vesicle transport from Endoplasmic Reticulum (ER) to Golgi apparatus, and is considered as the way for cargoes such as proteins to leave the ER. COPII has been very well studied over the last thirty years, however many critical steps in bud formation, protein mechanics and vesicle scission remain unclear.

This project focus in the deformation of ER membrane during COPII vesicle formation and the role of lipids. We have observed that certain proteins involved in lipid metabolism can rescue mutant phenotypes in the formation of COPII vesicles. Particularly, we have found that *sec12-4*, a mutation that affects COPII proteins recruitment, can be rescued by changes in the levels of certain lipid species. The accumulation of these lipids in membranes is thought to induce changes in the local curvature of the lipid bilayer. This finding highlight the importance that lipids may play the formation of the vesicle, where membrane deformation and scission are central tasks during the formation of a mature vesicle.

We follow a lipidomic and biophysical approaches, as we are interested in which lipids are involved in the formation of a vesicle and what is the role they are playing in the membrane. Therefore, we show results from mass spectrometry, *in vitro* and *in vivo* assays where we explore variations in lipid levels and its effect in the formation of COPII vesicles.

P11r-33
Trk1 is a salt-tolerance determinant in the yeast *Hansenula polymorpha*

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The study of the yeast *Hansenula polymorpha*, a member of the *Saccharomycetaceae*, has applied and basic interest for different reasons such as their ability to use methanol and nitrate as carbon and nitrogen sources respectively. Our group, in collaboration with Dr Siverio (ULL, Spain), has performed an initial study of potassium homeostasis in this yeast. We have identified two potassium transporters in the plasma membrane, Hak1 and Trk1, and we have proposed that Hak1 functions as a transporter with high affinity, which is necessary for growth at low concentrations of potassium [1].

In this work we have studied the role of Trk1 in *H. polymorpha*. By using wild type and single *trk1* mutant, we analyzed growth, cation contents, fluxes, relative membrane potential and volume in cells under different external conditions. Our results show that *trk1* mutant is more salt sensitive than wild type and that it accumulates more sodium and lithium due to increased influx processes. In summary, the results indicate that, while Trk1 does not seem to be a relevant potassium transporter for the cell, it has a fundamental role in salt tolerance, which is consistent with the idea that the TRK transporters may have additional and/or different functions to potassium transport [2].

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P11-34
Functional significance of conserved cysteines in the arsenic/antimony antiporter Acr3 from budding yeast

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The Acr3 permease from the yeast *Saccharomyces cerevisiae* is a prototype member of the arsenical resistance-3 (Acr3) family of transporters, which are found in all domains of life. Remarkably little is known about molecular mechanism of metalloid translocation via Acr3. Members of the Acr3 family exhibit ten-transmembrane topology and lack any known sequence signatures, which could suggest mechanism of metalloid flow. It has been recently revealed that a single cysteine residue (Cys129) in the *C*orynebacterium *glutamicum* Acr3, which is located in the fourth transmembrane span and conserved in all members of the Acr3 family, is indispensable for As(III) efflux. We have found that the mutation of this highly conserved residue (C151A) in the *S. cerevisiae* Acr3 also led to a complete loss of transport function. This strongly suggests that interactions between As(III) and a thiol group of this residue is required to activate transport through Acr3. In addition, we mutagenized the remaining three cysteine residues C192, C283, and C344, located also in the transmembrane spans of Acr3, and found that in contrast to the C151A mutant, these mutant forms of Acr3 were able to complement metalloid sensitivity of *ACR3* deletion yeast strain and showed only a slight decrease of efflux activity. Although, all cysteine mutants of Acr3 were expressed at the wild type level, four of them, C90A, C169A, C318A and C333A, were not properly secreted to the plasma membrane and accumulated in the intracellular membranes, suggesting that these residues are required for the ER exit. C90, C169, C318, and C333 are located in the intracellular loops and might be subjected to posttranslational modifications, like palmitoylation, which are required for membrane association, protein stability and protein trafficking.

P11-35
ABC transporter facilitates sequestration of plant glucosides in leaf beetle larvae

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ATP-binding cassette (ABC) tranporters are most intensively studied in the area of pharmacology and medicine but little is known about their crucial role in the defense of leaf beetle larvae. During the course of evolution, larvae of the subtribe Chrysomelina have evolved specialized defensive glands in which specific host plant derived glucosides are enzymatically converted and stored. This universal phenomenon has been termed sequestration and results in a toxic chemical cocktail, released by the larvae in case of an attack by a predator. However, none of the transport proteins essentially involved in these sophisticated import/export sequestration processes has been described to date.

We identified an ABCC4-like transporter CpMRP, primarily tranccribed in the exocrine glands, which acts as a pivotal modulator in the defensive system of *Chrysomela populi*. Further immunohistological studies revealed that CpMRP is localized in intracellular membranes of the glandular cells. RNAi-silencing of CpMRP *in vivo* results in a defenseless phenotype incapable of secreting the defensive compounds and is linked to aberrant vesicle morphology. By using the *Xenopus laevis* oocyte expression system, various plant glucosides could be verified via HPLC-MS to serve as substrates of CpMRP *in vitro*. In general, the glucoside specifity of CpMRP is low, as long as the sugar moiety consists of glucose. However, previous feeding experiments indicate the existence of a yet undescribed, highly specific glucoside transporter in the outer membrane of glandular cells.

With these findings we characterize one key player in the transport network of leaf beetle defense. ABC transporters may generally be involved in sequestration processes of herbivores/counter plant chemical defense and are possibly required for evolutionary flexible defense strategies in leaf beetle. Knocking out herbivore's defense could open a promising approach for future strategies in crop plant protection.

P12 – Metabolic Regulation

P12m-1
StarD5, an intracellular cholesterol transport protein, is regulated by the ER-stress: A possible mechanism to reduce ER free cholesterol accumulation

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StarD5 belongs to the START (steroidogenic acute regulatory lipid transfer) domain family of proteins. It is able to bind cholesterol, is a cytosolic protein and its mRNA expression is induced during Endoplasmic Reticulum (ER) stress. This suggests a role of StarD5 in intracellular lipid transport and homeostasis. However, its function remains poorly defined. This study's objective was to characterize StarD5 expression during ER-stress so as to pursue its function.
Methods: mRNA levels were measured following 3T3-L1 cell transfection with selective ER-stress transcription factors XBP-1s, ATF6 and ATF4, or by a more general ER-stress inducer, Thapsigargin(Tg). Filipin staining and immunocytochemistry were used to determine cellular free cholesterol and StarD5 localization. The presence of possible ER-stress sequence elements in the promoter region of the STARD5 gene and its mRNA stability during ER-stress were determined.
Results: Tg induced ER stress increased both intracellular free cholesterol and StarD5 mRNA levels by increasing its expression and mRNA stabilization. Furthermore, there was increased co-localization of StarD5 with the ER. Only overexpression of the ER stress transcription factor XBP-1s was able to induce StarD5 mRNA expression.
Conclusions: The ability of StarD5 to bind cholesterol coupled with its responsiveness to ER-stress, allow us to hypothesize a role for StarD5 in the maintenance of intracellular cholesterol levels under ER-stress conditions. Its mRNA stabilization and up-regulation by XBP-1s during the cell protective phase of the ER-stress suggests a role for StarD5 in the non-vesicular intracellular transport of cholesterol from the ER to other membranes in order to lower the amount of cholesterol that accumulates in the ER during ER-stress.

P12-2
Systems biology approaches to metabolic engineering in Streptomycetes: the Phosphoenolpyruvate-Pyruvate-Oxaloacetate node of primary metabolism

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Streptomycetes are important producer of bioactive secondary metabolites for use as antibiotics, immunosuppressants or anti-cancer drugs. One group of particular interest is the polyketides, which are biosynthesised through repeated condensation of acetyl-CoA units, through claisen-type reactions. Despite several decades of academic and industrial research on different species of Streptomycetes, the production yield of certain products remains relatively low, therefore an alternative for classic strain development methods is of need. A more rational approach such as systems biology may offer potential in this field of work. As polyketides are synthesized from precursor molecules originated in the central carbon metabolism in which the Phosphoenolpyruvate-Pyruvate-Oxaloacetate (PEP-PYR-OAA) node is a major control point of carbon flux and offers significant potential to investigate using systems biology approaches. A transposon-mutagensis approach has been used to disrupt all the genes contributing to the PEP-PYR-OAA node and each has been analysed for the influence on antibiotic yield to identify key enzymes, using actinorhodin from *Streptomyces coelicolor* A3(2) as a model polyketide. Many of the gene disruptions exhibit an antibiotic phenotype, but most dominant are the three paralogous pyruvate dehydrogenase complexes. Data will be presented on enzymatic analysis and further characterisation of the mutants.

P12-3
Yeast lipid homeostasis – new insights from a lipidomic screening of kinases and phosphatases

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Lipids are essential eukaryotic cellular constituents with multiple roles in cellular processes. The major lipid components in eukaryotic cells are glycerophospholipids, sphingolipids and sterols, whose molecular structures present a remarkable complexity. The cell's lipid composition is maintained by coordinated pathways between glycerophospholipids, sphingolipids and sterols. This interconnected network constitutes a dynamic biological core that allows cells not only to adapt their lipid profile to environmental challenges, but also to sense the levels of the different lipids and adjust their lipid composition to preserve cellular functions. Protein phosphorylation is a major regulatory mechanism that controls many basic signalling cellular processes in cells. Although some enzymes of lipid metabolism have been already described to be regulated by phosphorylation, a systematic analysis of how kinases and phosphatases affect lipid homeostasis was not done yet. With a few hundreds of lipid species, and sharing conserved metabolic pathways with mammals, the yeast *Saccharomyces cerevisiae* represents a useful model organism for studying lipid homeostasis. In this work, we performed a systematic and semi-quantitative lipid analysis of 130 yeast strains with gene deletions for kinases and phosphatases. By multiple-reaction-monitoring mass spectrometry (MRM-MS) we quantified lipids from the major classes in yeast, revealing lipidome wide changes in several mutants. Interestingly, the mutant with the largest extent of lipid alterations was SNF1, which is the central regulator of energy homeostasis in eukaryotes. By analysis of similarity we observed distinct clusters of mutants which presented alterations in the fatty acid chain length composition in phospholipids, ceramides and sphingolipds. Among those, there were kinases and phosphatases that are components of common signaling pathways, i.e. nutrient sensing pathways suggesting an underlying signaling mechanism for the observed changes. Taken together, the results show new connections on nutrient sensors, reinforced previously reported findings in the regulation of lipid metabolism, and also present new candidates for regulation of lipid homeostasis.

P12-4
A role for *Dun1* in the regulation of yeast ribonucleotide reductase in response to iron deficiency

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Ribonucleotide reductase (RNR) is an essential enzyme required for DNA synthesis and repair. In *Saccharomyces cerevisiae* multiple mechanisms, including the subcellular redistribution of the Rnr2-Rnr4 small RNR subunit from the nucleus to the cytoplasm and the degradation of the Rnr1-Rnr1 large RNR subunit inhibitor Sml1, regulate in a Mec1/Rad53/Dun1-dependent manner RNR function during the progress of the cell cycle and in response to genotoxic stresses. Although iron is necessary for class Ia RNR activity, little was known about the mechanisms that control RNR in response to iron deficiency. We have recently shown that yeast cells regulate RNR function during iron deficiency by promoting the redistribution of the RNR small subunit to the cytoplasm in a Mec1/Rad53-independent manner (Sanvisens et al., *Mol. Cell*, 2011, 44: 759–769). Instead, the iron-regulated Cth1/Cth2 mRNA-binding proteins specifically interact with the *WTM1* mRNA in response to iron scarcity and promote its degradation. The resulting decrease in the nuclear-anchoring Wtm1 protein levels leads to the redistribution of the Rnr2-Rnr4 heterodimer to the cytoplasm, where it assembles with Rnr1 as an active RNR complex and increases deoxyribonucleoside triphosphate levels. We show now that Sml1 protein levels decrease during iron deprivation. We have observed that this downregulation is independent of Cth1/Cth2, but depends on the Dun1 protein kinase. Furthermore, we have observed that cells lacking *DUN1* or expressing the *DUN1-D328A* mutant allele display significant defects in Rnr2-Rnr4 redistribution to the cytoplasm in response to low iron. Taken together, these results suggest that Dun1, but not Mec1 or Rad53, participates in the control of RNR function in response to iron deficiency.

P12-5
Molecular characterization of the genes *GPD1* and *GPP1* from the yeast *Kluyveromyces lactis*

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Yeasts, as all organisms, are able to react to stress situations by activating specific signaling pathways. For example, osmotic stress is counteracted by the accumulation of intracellular glycerol as a compatible solute. This is achieved by an increased activity of enzymes which divert the glycolytic flux from triosephosphates. Signaling in this context is mediated by the HOG pathway (High Osmolarity Glycerol), as one of at least four MAPK pathways (for Mitogen Activated Protein Kinase) in yeast. In *S. cerevisiae* two glycerol-3-phosphate dehydrogenases, encoded by *GPD1* and *GPD2*, can convert dihydroxyacetone phosphate to glycerol-3-phosphate, from which glycerol is produced by the irreversible reaction of two phosphatases encoded by *GPP1* and *GPP2*. In contrast, the milk yeast *Kluyveromyces lactis* disposes of only one gene for each of the corresponding enzymes, thus being much less redundant.

We therefore started with a molecular characterization of the glycerol biosynthesis pathway in *K. lactis*. In a first step, strains carrying deletions in the genes encoding KlGpd1 and KlGpp1 were constructed and analyzed for their phenotypes. We demonstrated that glycerol synthesis is essential for the survival of this yeast both under osmotic stress and under anaerobic growth conditions. However, in contrast to baker's yeast, in *K. lactis* the pathway is neither activated by heat stress nor by cell wall stress, e.g. caused by compounds such as Calcofluor white. Accordingly osmolabile phenotype was not suppressed by overexpression of several genes encoding components of the CWI pathway. Heterologous expression studies showed that the genes *KlGPD1* and *KlGPP1* complement the respective defects in *S. cerevisiae* mutants.

In order to investigate the connected signaling pathway in *K. lactis* in more detail, we also isolated the gene encoding KlHog1, the central MAPK of the HOG pathway, and constructed a null mutant. The deduced amino acid sequence shares 80% identity to its ScHog1 counterpart. As expected, the *Klhog1* deletion mutant displays sensitivity to salt and high osmolarity stress. We started with the investigation of the expression of putative target genes of the HOG pathway in *K. lactis* by fusing their promoters to *lacZ* reporter constructs. The first results will be presented.

P12-6
Regulation of carotenoid accumulation in *Dunaliella salina* by light-emitting diode lighting along with adaptive laboratory evolution

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There is a particularly high interest to derive carotenoids such as β-carotene and lutein from higher plants and algae for the global market. It's well known that β-carotene can be overproduced in *Dunaliella salina* in response to stressful light conditions. However, little is known about the effects of light quality on carotenoid metabolism, e.g. red light and/or blue light with narrow spectra. We have studied the effect of red LED lighting on growth rate and biomass yield and identified the optimal photon flux for *D. salina* growth. We found that the major carotenoids changed in parallel to the chlorophyll b content and that increasing the photon stress of red light alone was not capable of up-regulating carotenoid accumulation due to serious photodamaging. A pathway proposed based on LC/MS data is consistent with the one proposed for caorotenoid metabolism in *Chlamydomonas reinhardtii*. We have found that combining red LED (75%) with blue LED (25%) allowed growth at a higher total photon flux. Long-term iterative stress with additional blue light led to increased β-carotene and lutein accumulation. This application of adaptive laboratory evolution (ALE) yielded adapted strains of *D. salina* with increased accumulation of carotenoids under combined blue and red light condition.

P12m-7
Pancreastatin, a chromogranin A-derived peptide impairs adipocyte differentiation

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Pancreastatin (PST) is one of the biologically active peptides derived from the proteolytic process of chromogranin A (CGA), a glycoprotein present in chromaffin and neuroendocrine cells. Many different reported effects have implicated PST in the modulation of energy metabolism, with a general counterregulatory effect to that of insulin. PST induces glycogenolysis in liver and lipolysis in adipocytes. Metabolic effects have also been found in humans, and we have found increased plasma PST levels in insulin resistant patients, suffering from hipertension or gestational diabetes. Moreover, these metabolic effects of PST have been confirmed in CGA knockout mice, which have increased insulin sensitivity in spite of the fact that they have increased fat mass. In this line, we raised the hypothesis that PST may inhibit adipocyte differentiation. Therefore we aimed to study the effect of PST on 3T3-L1 adipocyte differentiation, triggered by insulin, IBMX and dexamethasone. We measured the expression of adipocyte differentiation markers, PPAR-gamma, CCAAT/enhancer-binding protein C-EBP-alfa, C-EBP-beta, which act as key transcription factors at an early stage of differentiation, followed by the expression of glucose transporter GLUT-4 by specific immunoblot and qRT-PCT, at days 4 and 6 and 8 after the differentiation. Intracellular lipid accumulation was determined by Sudan II staining during adipocyte differentiation. We have found that 100 nM PST inhibits the differentiation of 3T3-L1 adipocytes, as assessed by Sudan II staining. This effect seems to be mediated by decreasing the expression of PPR-gamma, C-EBP-alfa and beta, and GLUT-4 down to less than half the amount of those found in control 3T3-L1 cells. These results demonstrate that PST has an inhibitory effect on adipocyte differentiation, and this effect may further contribute to the insulin counterregulatory role of PST.

P12-8
GRK2 controls functionality of brown adipose tissue

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Introduction: Obesity is a major health problem and an important risk factor for the development of multiple disorders. Brown adipose tissue (BAT) is involved in the control of body temperature and plays an important role in regulating energy balance, so that alterations in this tissue have been associated with obesity and type 2 diabetes. Previous studies in our group have revealed that down-regulation of GRK2 decreases age-related adiposity, but the physiological and molecular mechanisms underlying this process remain unclear. In this work, we evaluate whether the lean phenotype of GRK2 hemizygous (+/−) mice results from a direct effect of GRK2 on energy homeostasis through the regulation of brown fat function.

Materials and Methods: We examined core temperature, energy expenditure, protein expression and phosphorylation levels, cold response and cold-induced changes in adipose tissues of wild-type and GRK2+/− mice. Moreover, we investigated the impact of GRK2 overexpression in brown adipocyte function and differentiation *in vitro*.

Results: Our study showed GRK2+/− mice displaying higher energy expenditure and a lower respiratory exchange ratio, consistent with these mice using more FAs as an energy source. Analysis of BAT from adult mice revealed a less deteriorated morphology associated with age in GRK2+/− compared to WT mice, which correlated with a higher basal core temperature. BAT from young GRK2+/− mice showed an increase in the expression of thermogenesis-related genes. Accordingly, hemizygous mice displayed better thermogenic capacity and exhibited a more oxidative phenotype in both BAT and WAT than WT littermates. Overexpression of GRK2 in brown adipocytes corroborated the negative effect of this kinase in brown adipocyte differentiation and function, the later mediated at least in part through an impairment of ERK1/2 activation upon β_3AR stimulation.

Conclusion: Our data point at GRK2 inhibition as a potential tool for the enhancement of brown fat activity, which may have important therapeutic implications for the treatment of obesity and associated disorders.

P12-9
GRK2 cardiac levels modulate cardioprotective gene expression patterns and sensitivity to insulin signaling pathway activation in adult mouse hearts

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G protein-coupled receptors (GPCRs) are essential regulators of cardiovascular physiopathology that are under the control of G protein-coupled receptor kinases (GRKs). In particular, the GRK2 isoform has emerged as a key regulator of cardiac function and dysfunction. The levels of cardiac GRK2 are increased during hypertension, heart failure and ischemia in humans, whereas genetic inhibition of GRK2 is cardioprotective in different animal models of these pathologies. However, the mechanistic basis for these effects is not fully understood. We have used adult

GRK2 hemizygous mice ($GRK2^{+/-}$) as a model to assess the effects of a sustained systemic inhibition of GRK2 in heart tissue with age. Using microarray RNA expression techniques we have compared the transcriptional profile of the cardiac tissue of wild-type and $GRK2^{+/-}$ C57BL6 mice of 4 or 9 months of age. We found that at 9 months of age GRK2 hemizygous mice show increased expression of relevant genes related to physiological hypertrophy compared to wild-type littermates, while the expression of key genes related to pathological heart hypertrophy are repressed. Consistently, these mice display changes in heart morphometry in the absence of fibrosis or dilation. Interestingly, in line with previous reports showing that $GRK2^{+/-}$ mice are more sensitive to insulin systemically, we find that insulin-dependent cardiac responses are altered in these mice. In sum, our study suggests that systemic reduction in GRK2 levels/function can facilitate the activation of certain cardioprotective routes, such as the insulin pathway, thus promoting a physiological hypertrophy-like gene expression pattern that would contribute to explain the cardioprotective outcome of GRK2 inhibition.

P12m-10
Effect of pleiotrophin in tissular lipid accumulation

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Overweight and obesity are defined as abnormal or excessive fat, and are risk factors for a number of chronic diseases, including diabetes, cancer and cardiovascular diseases. Recent studies have shown that pleiotrophin, an heparin-binding cytokine, may play a role in adipogenesis.

Thus, the aim of the present study was to characterize the effects of pleiotrophin disruption on lipid accumulation, in a dominant-negative pleiotrophin mutant mice model. For this purpose, we analyzed plasmatic and tissular parameters in 3, 6 and 12 months old female $PTN^{+/+}$ (WT) and $PTN^{-/-}$ (KO) mice.

Fat depots, liver, heart and body weight were significantly lower in $PTN^{-/-}$ than in WT mice at 3, 6 and 12 months of age. Analysis of lipid content in liver revealed that disruption of pleitrophin is associated with a decreased lipid content in liver. Circulating triglycerides were also lower in $PTN^{-/-}$ than in WT animals.

Our results suggest that pleitrophin could be implicated in tissular lipid accumulation, protecting against overweight and ectopic lipid accumulation associated with aging.

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P12-11
Bile acids and probiotics could help treating diabetes

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Introduction: Diabetic patients suffer complications due to disturbed physiological and biochemical processes associated with the disease including disturbed bile acids production and microfloral composition. Aim of this study was to examine the effect of pre-treatment with probiotics followed by the oral administration of gliclazide and monoketocholic acid (MKC), on blood glucose (BG) and gliclazide pharmacokinetics in type 1 diabetes (T1D) rat model.

Methods: The four out of eight groups of male Wister rats were injected alloxan intravenously inducing T1D. One healthy and one diabetic group were gavaged with probiotic mixture twice daily for three days. Upon probiotic treatment, a single dose of gliclazide + MKC (20 and 4 mg/kg, respectively) was administered either orally or intravenously. Blood samples were then collected at different time points after the dose. Gliclazide concentration in serum was measured by HPLC.

Results: Probiotic treatment significantly reduced BG in diabetic rats which was additionally reduced after gliclazide + MKC oral dose. In probiotic treated healthy rats, gliclazide bioavailability was the lowest. In contrast, in probiotic treated diabetic rats, gliclazide bioavailability was higher than in untreated diabetic rats. In untreated healthy rats, the bioavailability of gliclazide increased for 68.51% compared when given with MKC, while, in probiotic treated diabetic rats, gliclazide bioavailability remained unchanged, suggesting a different effect of MKC on gliclazide pharmacokinetics in diabetic animals.

Conclusions: MKC, with a hypoglycemic effect when given orally to T1D rats, and probiotics, with their beneficial effects, suggest that multidrug approach to treating diabetes could be useful as them being potential adjuvant treatments.

Keywords: Type 1 diabetes; bile acid; probiotics; gliclazide
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P12-12
The association of glycolytic enzymes from *Saccharomyces Cerevisiae* is stabilized by actin

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The cell contains constant concentrations of solutes and macromolecules except during stress, when compatible solutes accumulate in the cytosol (Ovádi J & Saks V. *Mol. Cell Biochem*. 2004; 256–257:5–12). Molecular crowding in the cell results in protein association that allows the channeling of intermediates and thus increasing metabolic efficiency (Srere PA. *Annu. Rev. Biochem*. 1987; 56:89–124). Multienzymatic complexes are anchored in a dynamic cytoskeleton. It is suggested that the efficiency of metabolism is due to metabolons, clusters of enzymes that perform in coordination (Minaschek G *et al. Eur J Cell Biol*. 1992; 58:418–28). Also, metabolons probably protect the enzymes from the deleterious effects of stress. The protection mechanism has not been explored. We decided to examine whether the association of different glycolytic enzymes with actin results in higher protein stability. Enzyme association among themselves and with the cytoskeleton was assessed by co-immunoprecipitation of actin and glycolytic enzymes. The stabilizing role of F-actin on the associated glycolytic enzymes and the protection against inhibition by a compatible solute (trehalose) was also tested. The participation of F-actin in metabolic stability was evaluated in the whole fermentation pathway which remained active at the trehalose concentrations that inhibit single enzymes. Enzyme protection against compatible solute accumulation is proposed as a novel property of metabolons.

P12-13
GLUT2$^{-/-}$ mice are resistant to age-induced accumulation of fat in the liver – an *in vivo* MRS study

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Introduction: Whole-body energy balance is strongly dependent on the hepatic handling of metabolic fuels, which is affected by the hepatic lipid content (HLC). With *in vivo* nuclear magnetic resonance spectroscopy (MRS) HLC can be measured non-invasively and longitudinally. The study of mice is relevant for the characterization of transgenic models that provide insight into disease mechanisms but comparing with bigger subjects, the reduced sample size remains a limiting factor for *in vivo* MRS experiments. To overcome this sensitivity issue, we performed liver MRS *in vivo* at high field (14.1T) in wild-type C57BL/6J mice (WT) and GLUT2$^{-/-}$ mice that display altered whole-body energy balance.
Methods: WT and GLUT2$^{-/-}$ mice reexpressing GLUT1 in the pancreatic β-cells to allow for survival and normal glucose-stimulated insulin secretion were studied at 4 months and 1 year of age. Non-fasted mice under isofluorane anesthesia were scanned in the supine position with a ^{1}H quadrature surface coil over the abdomen. MRS measurements were performed in a horizontal bore 14.1T-26 cm magnet. Multi-slice gradient echo images were acquired for anatomical identification of the liver. Localized, respiration-gated ^{1}H-MR spectra were acquired from a 10–15 μl voxel with STEAM with and without water suppression. HLC was estimated as the T2-corrected area of 1.3 ppm-lipid resonance relative to that of the water plus 1.3 ppm-lipid.
Results/Discussion: Highly sensitive MRS at 14.1T allowed to accurately quantifying HLC in short experiments in mice. Suppression of the water signal revealed fatty-acyl resonances reflecting the lipid saturation profile. At 4 months, HLC was similar between WT and GLUT2$^{-/-}$. HLC increased significantly in aged WT mice but remained low in aged GLUT2$^{-/-}$. Also, the body weight of aged GLUT2$^{-/-}$ was slightly lower than that of WT. Low HLC in aged GLUT2$^{-/-}$ mice probably results from a defect on intra-hepatic pathway fluxes due to GLUT2 ablation in the liver. In addition, it may reflect altered utilization of metabolic substrates due to inadequate whole-body blood glucose sensing.

P12-14
Growth and osmolyte distribution of a moderately halophilic microorganism in the presence of phenol

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Many industries, such as petroleum refining, petrochemical, pharmaceutical, leather, textile and, phenol-formaldehyde generate thousands of liters of wastewater discharges with phenolic compounds every year. Unfortunately, these phenolic compounds are hazardous pollutants that are toxic even at relatively low concentrations. Due to their ability to grow optimally between 0.85 and 3.4 M NaCl moderately halophilic bacteria are offered as potential candidates to be used for phenol removal from saline wastes. Under stress conditions such microorganisms commonly accumulate osmolytes, which are small organic compounds that improve protein stability. In this study, we have primarily focused on the adaptation and growth of the gram negative, moderately halophilic *Halomonas* sp. AAD12 to the presence of various NaCl and phenol concentrations. There was significant cell growth in Brown media supplemented with 5% NaCl up to a phenol concentration of 800 mg/l. Further increase in phenol concentration inhibited growth in this salinity. When NaCl concentration was kept at 10%, there was measurable cell growth only in the presence of 200 mg/l phenol. Cells could not survive even in the presence of 200 mg/l when NaCl concentration was increased to 15% and 20%. Finally, we proposed that osmolytes' accumulation strategy might be significant in adaptation to phenol since these organic molecules are synthesized as a response to stress. To this end, cultures have been sampled in mid- and late-exponential phases and the osmolytes were extracted. ^{1}H-NMR spectroscopy had been used to identify and quantify the distribution of the major osmolytes; ectoine, hydroxyl-ectoine and proline.

P12r-15
Hepatocyte Growth Factor and skeletal muscle insulin resistance

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Insulin resistance (IR) is a hallmark of diabetes and obesity, which lead to abnormal glucose metabolism as a result of impaired metabolic response to insulin. The mechanism underlying the etiology of IR remains incompletely understood. Excessive production of pro-inflamamatory cytokines by the adipose tissue is linked to IR. Hepatocyte Growth Factor (HGF) is an adipocytokine elevated in obese subjects, which activates glucose transport and metabolism in rodent myotubes *in vitro*. However, the role of HGF in the etiology of the IR remains unknown. In this work, we hypothesize that HGF improves muscle glucose metabolism and ameliorates skeletal muscle insulin resistance *in vivo*. To this end, we developed a conditional transgenic mouse of HGF in the skeletal muscle (SKM-HGF). HGF expression is up-regulated in the skeletal muscle by administration of doxycycline (DOX). Oral administration of DOX (1 mg/ml) for 1 month elevated by threefold HGF skeletal muscle levels in SKM-HGF mice compared with control mice. However, fasting and non-fasting plasma glucose and insulin levels, glucose tolerance and insulin tolerance were similar between SKM-HGF mice and control mice. To further investigate the role of HGF in the IR skeletal muscle, control and SKM-HGF mice were fed high fat diet (HFD) maintaining the administration of DOX for 3 months. As expected, control mice fed HFD showed glucose intolerance, hyperglycemia and hyperinsulinemia compared to wild type mice fed chow diet. However, fasting and non-fasting plasma glucose and insulin levels, glucose tolerance and insulin tolerance were similar between SKM-HGF mice and control mice fed HFD. Interestingly, overexpression of HGF in skeletal muscle resulted in enhanced phosphorylation of protein kinase B upon insulin stimulation *in vivo*. In conclusion, our data suggests that HGF is a biomarker of obesity in humans and it is not involved in the etiology of skeletal muscle IR.

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P12-16
Hepatic gluconeogenesis: a joint effort of cytosolic and mitochondrial PEPCK

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Hepatic gluconeogenesis helps maintaining systemic energy homeostasis by compensating for discontinuities in nutrient supply. Liver specific deletion of cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) abolishes gluconeogenesis from mitochondrial substrates, dysregulates lipid metabolism and severely blunts the TCA cycle. Although PEPCK-C is the dominant PEPCK isoform in mice, humans express the cytosolic and mitochondrial isoforms equally. Despite the clear relevance to human physiology, the role of the mitochondrial isozyme (PEPCK-M) remains unknown. Here, we test the significance of PEPCK-M in gluconeogenesis and TCA cycle function by overexpressing PEPCK-M in liver-specific PEPCK-C knockout mice. These mice were examined by a combination of tracer studies and molecular biology techniques. Assessment of hepatic flux using ^{2}H and ^{13}C tracers in combination with NMR isotopomer analysis revealed that PEPCK-M expression partially rescued defects in gluconeogenesis and TCA cycle function impaired by PEPCK-C deletion. In contrast, ~10% re-expression of PEPCK-C normalized flux, physiology and exercise capacity of liver KO mice. Examination of PEPCK-M in presence of PEPCK-C revealed that the mitochondrial isozyme amplifies gluconeogenic capacity of PEPCK-C, suggesting autonomous regulation of oxaloacetate to phosphoenolpyruvate fluxes by the individual isoforms. We conclude that PEPCK-M has gluconeogenic potential *per se*, and facilitates gluconeogenesis from PEPCK-C via effects on mitochondrial cataplerosis. These findings provide critical insights into the potential gluconeogenic and cataplerotic role of PEPCK-M in human and other species, and its possible involvement in pathological states such as diabetes or obesity.

P12-17
Free fatty acids and acylcarnitines profiling in rat to elucidate drug-induced steatosis

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Microvesicular steatosis is a chronic hepatocellular change associated with certain xenobiotics and with an inhibition of mitochondrial fatty acid β-oxidation (mitFAO) [1,2]. We addressed the impairment of mitFAO related with valproic acid (VPA), one of the most important anti-convulsive drugs [1,3].

Aim: To investigate the interference of this drug with mitFAO *in vivo* through the analysis of plasma free fatty acids (FFA), carnitine and acylcarnitine in rat liver; to elucidate the potential secondary carnitine deficiency as an adverse effect induced by the drug.

Methods: The analysis of around thirty individual FFA was achieved using GC-MS in the SIM detection mode. A comprehensive study of free carnitine and around forty different acylcarnitines was undertaken in liver tissues using tandem mass spectrometry (ESI-MS/MS). The samples were obtained from Wistar rats subjected to a single injection or a subchronic regimen with VPA.

Results: In VPA-treated animals a significant increase ($p < 0.05$) in the plasma levels of dicarboxylic acids was found as compared to controls. There was a significant dose-related increase of free carnitine and total acylcarnitine fraction in the liver of rats treated with a single VPA injection. Acylcarnitine levels were normal in livers taken from rats on the subchronic treatment except for malonyl-, isovaleryl-, 3-hydroxyisovaleryl-, adipoyl-, *cis*-4-decenoyl- and decanoylcarnitine that were significantly increased.

Discussion: Activation of ω-oxidation seems to be an important pathway in rats, as in humans, to rescue mitochondrial FFA oxidation inhibited by VPA. Results show that free carnitine is not depleted in liver of VPA-treated rats. The induction of endogenous carnitine biosynthesis in the liver may play an important role as an adaptation mechanism to drug-induced stress on mitochondrial metabolism.

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P12-18
Quercetin fluorescence reveals the open probability of the RyR3 Ca^{2+} channel in intact cells

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The fluorescent properties of the natural flavonoid quercetin in human leukemia Jurkat T-cells were studied by means of spectrofluorimetric measurements. Intracellular quercetin displayed a dominant emission maximum at ~540 nm and two well-defined excitation maxima, at ~380 and ~440 nm, respectively. Our data suggest that these dual-excitation bands correspond to two distinct molecular configurations of a quercetin-bound protein, which are sensitive to the cytosolic Ca^{2+} concentration. Here we present strong evidence that the ratio of the fluorescence emitted by intracellular quercetin at 540 nm upon excitation at 380/440 nm reflects the open probability of the RyR3/Ca^{2+} release channel in its native environment, the endoplasmic reticulum membrane. The kinetics of the Ca^{2+} release signal induced by high levels of quercetin in intact cells and that of F380/F440 were strongly correlated. The RyR3 inhibitor, ruthenium red, depressed consistently the QC-induced Ca^{2+} signal. The effect of Ca^{2+}, quercetin, dantrolene and Mg^{2+} on F380/F440 was also investigated in permeabilized cells. A novel regulatory mechanism was identified by which the RyR3 channel activity under physiological conditions is partially suppressed (hindered channel) whereas the channel becomes nearly fully activated after exposure to millimolar concentrations of bulk cytosolic Ca^{2+} and subsequent chelation of Ca^{2+} (rectified channel). Upon rectification, the bell-shaped dependence of the F380/F440 ratio on the cytosolic Ca^{2+} concentration was remarkably similar to that of the RyR3 open probability reported from bilayer experiments. All these findings shed light on the physiological regulation of the

$RyR3/Ca^{2+}$ release channel and advance the first direct method to assess its activity *in situ*.

P12r-19
Transcription elongation factor TFIIS.1 gene is regulated by farnesoid X receptor

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Continuous exposure to an excess of bile acids can induce reactive oxygen species production and oxidative DNA damage. The blockage of transcription elongation by RNA polymerase II (RNAPII) at oxidative DNA lesions such as 8-oxo-2′-deoxyguanosine (8-oxodG) compromises accurate transcription and can trigger cell death. Transcription elongation factor TFIIS, an important player in the transcription-coupled repair, enables RNAPII to bypass 8-oxodG lesion and provides an mRNA proof-reading mechanism. We have discovered by genomic differential expression analysis that human TFIIS.1 gene (TCEA2) is regulated by the farnesoid X receptor (FXR), a bile acid-activated nuclear receptor. FXR ligands strongly induced the expression of TCEA2 in human hepatoma cells (Huh7, HepG2), primary hepatocytes, vascular smooth muscle cells, renal tubule cells (HK-2), and breast cancer cells (MDA-MB-231). siRNA-mediated depletion of FXR in Huh7 cells eliminated the up-regulation of TCEA2 by natural (CDCA) and synthetic (GW4064) FXR agonists. In contrast, adenovirus-mediated overexpression of constitutively active FXR increased TCEA2 mRNA levels. Actinomycin D treatments blocked the response to FXR activators. Accordingly, reporter gene activation, ChIP and EMSA analysis identified a functional intronic FXR response element in TCEA2 gene. In addition, mice fed a chow diet supplemented with cholic acid or injected with GW4064 showed increased hepatic Tcea2 expression, whereas no such induction was observed in agonist-treated FXR null mice. We believe that the induction of TFSII.1 expression by FXR may be a mechanism of protection against cytotoxicity due to oxidative DNA damage induced by bile acids.

P12r-20
Estrogen related receptor (ERR)α-dependent activation of carnitine palmitoyltransferase 2 (CPT2) by PGC-1s

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The carnitine palmitoyltransferase 2 (CPT2) is one of several closely related mitochondrial-membrane carrier proteins that shuttle substrates between cytosol and the intramitochondrial matrix space. This protein is located in the inner mitochondrial membrane and converts acylcarnitine to acyl-CoA for their oxidation by the mitochondrial fatty acid-oxidation pathway. Carnitine palmitoyltransferase 2 deficiency can cause a variety of pathological conditions such as hypoketotic hypoglycemia, cardiac arrest, hepatomegaly, hepatic dysfunction and muscle weakness, and it may be lethal in newborn and infants. Here we report that the expression of the CPT2 gene is induced in mouse skeletal muscle after a 24 h starvation period. To gain insight into the control of CPT2 gene expression, we examined the transcriptional regulation of the mouse CPT2 gene. We show that the 5′-flanking region of this gene is transcriptionally active and contains a consensus sequence for the estrogen-related receptor (ERR) a member of the nuclear receptor family of transcription factors. This sequence is able to bind ERRα and is necessary for the activation of CPT2 expression by the PGC-1/ERR axis. We also show that the inverse agonist of ERRα XTC790 specifically blocks the activation of CPT2 by PGC-1s in C2C12 cells.

P12-21
Involvement of mitochondrial redoxins in iron metabolism

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Yeast glutarredoxin-2 (Grx2p) is a dithiol protein with two different isoforms localized in mitochondria and cytosol, respectively. Peroxiredoxin-1(Prx1p) is a mitochondrial enzyme belonging to 1-Cys type of Prx. Both proteins are functionally related. Recently we have shown that, under fementative conditions, lack of Prx1p up-regulates the ATF1-dependent iron regulon genes but simultaneous absence of Grx2p abolishes this activation. In this report, we have studied the role of Grx2 and Prx1 on iron metabolism under respiratory conditions when mitochondrial function is essential for growth. Cells lacking Grx2p or Prx1p or both, grow normally on glucose, but the double mutant grows very slowly on glycerol. All mutants show increased levels of total Fe under both conditions. Cytosolic Fe increased, mitochondrial Fe decreased and SIT1 gene was up-regulated while AFT1 dependent genes were not, in the GRX2 mutant growing on glycerol. At the same time, mitochondrial Fe-S biogenesis was optimal but DRE2 was down-regulated in this mutant. Under the same conditions, mitochondrial Fe content diminished, and ARN1 and DRE2 genes were up-regulated but FET3 was not in the PRX1 mutant. These results show a clear correlation between Grx2p, Prx1p, AFT1 independent iron uptake and mitochondrial and cytosolic iron-sulfur biogenesis, which will be discussed.

P12m-22
Regulation of FGF21 under amino acid deprivation

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Responses to starvation in higher eukaryotes are initiated in part by hormonal changes in response to decreasing levels not only of glucose, but also of amino acids in the blood. The response to amino acid deprivation in mammals is primarily characterized by both repression of protein synthesis and upregulation of amino acid biosynthesis and transporters. One of the signal transduction pathways that are triggered in response to protein or amino acid starvation is the GCN2/eIF2α/ATF4 or the amino acid response (AAR) signal transduction pathways. FGF21, a member of a family of atypical fibroblast growth factors (FGFs), is highly induced upon fasting, and potently regulate peripheral glucose tolerance and hepatic lipid metabolism. It has been proposed that FGF21 could act subsequent to glucagon during nutritional deprivation to obtain and coordinate diverse aspects of the adaptive starvation response. We have uncover that the fasting hormone FGF21 was induced in the fed state in the liver of mice

deprived from leucine, a novel regulatory pathway of regulation of FGF21 gene expression. We have extensively characterized the human FGF21 gene as a target gene for ATF4, and we have identified two functional conserved ATF4-binding sequences in the 5′ regulatory region of the human FGF21 gene. Additionally to amino acid deprivation, FGF21 expression was increased upon proteasome inhibition and ER stress induction in HepG2 cells. The self-limiting cycle of ATF4-dependent transcription, previously described for several AARE-containing genes, was also observed for FGF21. Some interesting coincidences between the leucine deprivation resulting phenotype and FGF21-dependent metabolic effects suggested FGF21 as the link between amino acid starvation and overall energy homeostasis.

P12-23
A novel mechanism regulates ribonucleotide reductase function in response to iron deficiency

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Iron is an essential micronutrient for all eukaryotes because it participates as a vital cofactor in multiple cellular processes including DNA synthesis. Ribonucleotide reductase (RNR) is an essential iron-dependent enzyme that catalyzes the reduction of ribonucleotides to the corresponding deoxy forms for DNA synthesis and repair. In the yeast *Saccharomyces cerevisiae* RNR activity is regulated by multiple mechanisms that ensure the high fidelity of DNA replication during normal cell-cycle progression and DNA repair upon genotoxic stress. Although iron is necessary all eukaryotic RNRs, little was known about the mechanisms that control RNR function in response to iron deficiency. In this work, we show that yeast cells control RNR function and deoxyribonucleoside triphosphate (dNTP) levels during iron deficiency by regulating the expression and subcellular localization of the Rnr2-Rnr4 small RNR subunit in a Mec1/Rad53-independent manner. Upon iron scarcity the iron-regulated Cth1 and Cth2 mRNA-binding proteins specifically interact with the *WTM1* mRNA and promote its degradation. The resulting decrease in the nuclear-anchoring Wtm1 protein levels leads to the redistribution of the Rnr2-Rnr4 heterodimer to the cytoplasm, where it assembles as an active RNR complex and increases dNTP levels. Thus, when iron is scarce, cells selectively optimize RNR function in detriment of other non-essential iron-dependent processes such as respiration, to allow DNA synthesis and repair.

P12r-24
The glycogen activity of R6, the main PP1 regulatory subunit in brain, is modulated by laforin-malin complex

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Lafora disease (LD, OMIM 254780) is a progressive myoclonus epilepsy characterized by a fatal neurological deterioration and the presence of inclusions of poor-branched glycogen, called Lafora bodies (LB). These LB suggest a disregulation on glycogen metabolism. R6 is one of the glycogen targeting subunits of type 1 protein phosphatase (PP1) and enhances glycogen synthesis by recruiting the phosphatase to its glycogenic substrates. We have studied the interaction between R6 with malin and laforin, the two proteins whose mutations cause Lafora disease, by different techniques. We have found a direct interaction between laforin and R6 by yeast two hybrid assays, corroborated *in vivo* by co-inmunoprecipitation in mouse N2a neuroblastoma cells and by microscopy using acceptor photobleaching FRET technique in this same cell line. As a consequence of this interaction we performed *in vivo* ubiquitination studies showing that R6 is ubiquitinated by malin in a laforin-dependent manner. We also reported that this discrete ubiquitination involved the introduction of mainly K63-linked ubiquitin chains, but also K48, by malin-laforin complex. In addition, expression of R6 in N2a cells produce a three-five fold increase in glycogen production, promoting its accumulation. The co-expression of malin and laforin supresses partially the up-regulation on glycogen synthesis through this post-traslational modification. We have found that R6, when overexpressed, is apparently forming granules in N2a cell line. With all these results we are beginning to understand the complex mechanism of R6 regulation.

P12-25
PFKFB3 regulation by p38 MAPK pathway

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Cellular stress activates multiple mitogen-activated protein kinase (MAPK) cascades and the transcription of immediate-early genes. Mitogen-activated protein kinase (MAPK) signalling occurs in response to almost any change in the extracellular or intracellular milieu that affects the metabolism of the cell, organ or the entire organism. Glycolytic flux is mainly controlled by 6-phosphofructo-1-kinase, with fructose-2,6-bisphosphate (Fru-2,6-P_2) being its most powerful allosteric activator. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) catalyzes the synthesis and degradation of Fru-2,6-P_2 and hence critically regulates carbohydrate metabolism. *PFKFB3* gene codifies for PFKFB3 isoenzyme which has been found overexpressed in proliferating cells and tumors. Here we analyse its mechanism of regulation by cellular stress in different types of cancer cells. We report that exposure of HeLa and T98G cells to anisomycin (a drug that activates p38 pathway), UV radiation, hydrogen peroxide and osmotic shock lead to a rapid increase in Fru-2,6-P_2 concentration and *PFKFB3* mRNA levels. Western blot results showed a short-term activation due to PFKFB3 isoenzyme phosphorylation. Transient transfection of HeLa cells with deleted gene promoter constructs allowed us to identify a serum response element (SRE) through which p38-MK2 pathway transactivates *PFKFB3* gene transcription. A dual mechanism affecting PFKFB3 protein and gene regulation operates in order to assure glycolysis in these cell types. An immediate early response through MAPK phosphorylation of PFKFB3 protein is followed by activation of mRNA transcription via *cis*-acting sequences on *PFKFB3* promoter.

P12-26
Increased fatty acid oxidation protects adipocytes from lipid-induced insulin resistance

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The pathophysiology of obesity-induced insulin resistance and type 2 diabetes is thought to be due to both ectopic fat deposi-

tion and adipose tissue inflammation. During obesity, adipocytes increase in number and size reaching their storage capacity limit that leads to adipocyte death and immune cell infiltration. We propose that an increase in the adipocyte fatty acid oxidation (FAO) rate could protect from obesity and insulin resistance by a decrease in the lipid content and inflammatory levels. Malonyl-CoA, derived from glucose metabolism and the first intermediate in lipogenesis, regulates FAO by inhibiting carnitine palmitoyltrasnferase 1 (CPT1), thus making this enzyme the rate-limiting step in mitochondrial FAO. Here, we overexpressed CPT1A and CPT1AM (a permanently active mutant form of CPT1A, insensitive to malonyl-CoA) in 3T3-L1 CAR1 adipocytes by adenovirus infection. In our conditions, CPT1A protein and mRNA levels increased by 6-, and 3-fold, respectively, both for CPT1A and CPT1AM expression, compared to control. Moreover, CPT1A-, and CPT1AM-expressing adipocytes showed increased FAO and decreased triglyceride content and expression of proinflammatory markers after palmitate incubation. Interestingly, CPT1A overexpression restored palmitate-induced impairment of insulin signaling (measured as pAKT/AKT). In conclusion our study shows a beneficial effect of CPT1A overexpression on fatty acid-induced insulin resistance in adipocytes. This points out to an increase in adipocyte FAO as a promising strategy for the treatment of the obesity-induced metabolic syndrome.

P12r-27
Differential pharmacologic properties of the two C75 enantiomers: (+)-C75 is a strong anorectic drug. (−)-C75 has antitumor activity

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C75 is a synthetic inhibitor of fatty acid synthase (FAS), reported to have anti-tumor activity *in vitro* and *in vivo*. However, it suppresses food intake and reduces body weight, thus limiting its use in cancer therapy. It has been postulated that the anorexic effect of C75 could be due to the hypothalamic inhibition of FAS or of the enzyme carnitine palmitoyltransferase (CPT) 1, through its coenzyme A derivative, C75-CoA. This would lead to the accumulation of their substrates, malonyl-CoA and long chain fatty acyl-CoA (LCFA-CoA), reported to be molecular signals of appetite and body weight regulation in hypothalamic neurons. Since initially synthesized, a racemic mixture of C75 (here (±)-C75) was used in all published studies. Nevertheless, the stereochemistry of any drug is likely to determine its biological activity. To analyze the possible differential pharmacological effect of the two enantiomers and develop more specific drugs, we prepared (+)-C75 and (−)-C75 by *de novo* synthesis. Our results indicate that each enantiomer showed stereoselectivity for its respective target: FAS and CPT1. (−)-C75 inhibited FAS activity *in vitro* and was cytotoxic against several tumour cell lines. Importantly, (−)-C75 did not affect food intake in rats, providing an advantage over (±)-C75 for use in cancer therapy. On the other hand, (+)-C75, once activated to (+)-C75-CoA, inhibited CPT1 *in vitro* and reduced food intake, body weight and hypothalamic CPT1 activity in rats. As expected, the effects of (±)-C75 are the sum of the two enantiomers. Altogether, these results indicate that the two C75 enantiomers show stereoselectivity for their respective targets, FAS and CPT1. The pharmacological effects of C75 can thus be separated, which may lead to more specific drugs for cancer and obesity.

P12-28
Hypothalamic over-expression of carnitine palmitoyltransferase 1A produces hyperphagia, overweight and insulin resistance in rats

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The hypothalamus is a specialized area of the brain with a key role in the regulation of energy homeostasis. In response to changes in metabolic status, hypothalamic neurons alter the expression of neuropeptides and neuromodulators in order to adjust food intake and energy expenditure. It is unclear how the expression of these molecules is regulated; however recent studies highlight the fatty acid metabolism pathway as a main character in hypothalamic control of feeding. Several studies have demonstrated that modulation of this pathway can regulate feeding behaviour by altering the hypothalamic pool of malonyl-CoA and/or LCFA-CoA, suggesting that these metabolites are signals of nutrient status able to modulate not only food intake but also glucose homeostasis. Carnitine palmitoyltransferase (CPT) 1 is the rate-limiting step in LCFA-CoA β-oxidation and its activity is physiologically inhibited by malonyl-CoA. To analyze the role of this protein in the control of feeding we used adeno-associated virus (AAV) to over-express a permanently active form of CPT1A (malonyl-CoA-insensitive CPT1A, here CPT1AM) in the hypothalamus of rats. The data presented demonstrate that a long-term increase in hypothalamic CPT1AM levels leads to central and peripheral responses producing hyperphagia, overweight and insulin resistance. CPT1AM over-expression also produced an increase in hypothalamic NPY receptor (NPY5R) and ghrelin receptor (GHS-R) mRNA levels, suggesting that CPT1AM-expressing animals present a greater response to orexigenic neuropeptides and hormones. Taking together, these data highlight the central role of hypothalamic CPT1A in the regulation of food consumption and glucose homeostasis.

P12-29
Involvement of lipid droplets in hepatic responses to lipopolysaccharide treatment in mice

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As part of the central roles that liver plays in organic homeostasis of lipids, hepatocytes can accommodate high quantities of lipids in cytoplasmic lipid droplets (LDs). They are organelles composed of a core of neutral lipids surrounded by a monolayer of amphipathic lipids and cholesterol, where proteins implicated in the assembly, the biogenesis and the regulation of lipolysis of LDs are embedded. Infection and inflammation induce important

changes in lipid metabolism, resulting in increased plasmatic free fatty acids, hypertriglyceridemia and altered HDL. On the other hand, augmented accumulation of LDs in immune cells has been observed in both clinical and experimental infectious conditions. Our objective in this work was to elucidate to what extent hepatic LDs are involved in the organic adaptations of lipid metabolism to endotoxemia. We characterized the lipid content and, for the first time to our knowledge, several enzymatic activities in LDs and subcelullar fractions of livers from mice treated with lipopolysaccharide (LPS), a well-characterized inducer of cytokine secretion commonly used to mimic infection both *in vivo* and *in vitro*. We also analyzed the expression of key genes involved in lipid management. Compared to control animals endotoxemic mice showed much lower lipid content in LDs with decreased cholesteryl ester molar fraction and higher diacylglycerol/triacylglycerol ratio. They also showed an overall increase of the neutral lipid biosynthetic capacity and differential modifications of triacylglycerol and diacylglycerol hydrolytic capacity. The mRNA of lipogenesis-related genes and genes involved in HDL formation was overexpressed. The alteration in the hepatic metabolism of neutral lipids in LPS-treated mice could be related to the channeled mobilization of triacylglycerol to VLDL assembly and to the induction of cholesterol export contributing to increased serum HDL.

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P12-30
High insulin levels are required for FAT/CD36 plasma membrane translocation and enhanced fatty acid uptake in obese Zucker rat hepatocytes

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In myocytes and adipocytes, insulin increases fatty acid translocase (FAT)/CD36 translocation to the plasma membrane (PM) enhancing fatty acid (FA) uptake. Evidence links increased hepatic FAT/CD36 protein amount and gene expression with hyperinsulinaemia in animal models and patients with fatty liver, but whether insulin regulates FAT/CD36 expression, amount, distribution and function in hepatocytes is currently unknown. To investigate this, FAT/CD36 protein content in isolated hepatocytes, subfractions of organelles and density-gradient isolated membrane subfractions was analyzed in obese and lean Zucker rats by western blotting, in liver sections by immunohistochemistry, and in hepatocytes by immunocytochemistry. The uptake of oleate and oleate incorporation into lipids was assessed in hepatocytes at short time-points (30–600 sec). We found that FAT/CD36 protein amount at the PM was higher in hepatocytes from obese rats than from lean controls. In obese rat hepatocytes, decreased cytoplasmatic content of FAT/CD36 and redistribution from low-to-middle to middle-to-high density subfractions of microsomes were found. Hallmarks of obese Zucker rat hepatocytes were increased amount of FAT/CD36 protein at the PM and enhanced FA uptake and incorporation into triglycerides, which were maintained only when exposed to hyperinsulinaemic conditions (80 mU/l).

Conclusion: High insulin levels are required for FAT/CD36 translocation to the PM in obese rat hepatocytes, to enhance FA uptake and triglyceride synthesis. These results suggest that the hyperinsulinaemia found in animal models and patients with insulin resistance and fatty liver might contribute to liver fat accumulation by inducing FAT/CD36 functional presence at the PM of hepatocytes.

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P12-31
Possible mechanism of metabolic regulation by sirtuins

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The nuclear-localized lysine deacetylase, Sirt6 is a member of the sirtuin (silent information regulator) family which is involved in regulation of metabolism, ageing, genome stability, and fat homeostasis. Sirt6 posseses enzymatic activity on Histone H3 and CtIP. SIRT6 knockout mice are characterized by a dramatic premature ageing phenotype and typically do not survive past 4 weeks, whereas SIRT6 overexpression in male mice significantly extends their lifespan. Of particular interest is the regulatory role Sirt6 may play in increasing lifespan of various organisms that are subjected to dietary (calorie) restriction. We have studied the relationship between Sirt6 expression in hepatocytes and important cellular metabolic regulators. Examination of adenosine phosphate levels together with protein expression studies support the existence of a complex regulatory network whereby Sirt6 activity is modulated by other factors that play a role in maintaining cellular energy balance.

P12m-32
Effects of high-glucose levels on lipid metabolism in human placenta

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Gestational diabetes mellitus (GDM) has significantly increased over the past decades and is serious public health concern worldwide. Epidemiological studies have shown that the main adverse perinatal outcome of GDM is macrosomia, which is characterized by fetal fat accretion and overgrowth. Interestingly, macrosomia increases the risk for developing metabolic diseases such as obesity and diabetes later in life. It has been proposed that placental lipid metabolism represents a regulatory step towards macrosomia. However, the mechanism by which GDM regulates placental lipid metabolism and translates into fetal adiposity remains obscure. In this work,we hypothesized that hyperglycemia, a hallmark of GDM, impairs the ability of placentas from diabetic women to oxidize fatty acids, leading to accumulation of placental triglycerides. We showed that mitochondrial fatty acid oxidation (FAO) was reduced by ~20% in placenta from women with GDM. Likewise, high-glucose levels significantly reduced by ~20% the FAO rate in placental explants from healthy woman. In parallel, high-glucose levels increased by ~40% esterification, whereas *de novo* fatty acid synthesis remained unchanged.

Finally, high-glucose levels raised by ~2-fold placental triglyceride content. In conclusion, we reveal an unrecognized role of maternal hyperglycemia on the regulation of placental fatty acid metabolism, shifting flux of fatty acids away from oxidation towards the esterification pathway, leading to accumulation of placental triglycerides, Finally, our data suggest that in pregnancies with GDM, hyperglycemia is a contributor factor to fetal adiposity through the regulation of placental lipid homeostasis.

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P12-33
Resveratrol enhances palmitate-induced HepG2 cell death through inhibition of stearoyl-CoA desaturase 1 expression and ER stress-induced apoptosis

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Endoplasmic reticulum (ER) stress is a situation characterized by the accumulation of unfolded protein in the ER. To cope with this situation, the cell activates UPR (Unfolded Protein Response) mechanisms. It has been reported that saturated fatty acids can induce cell death through ER stress activation. Additionally, it has also been shown that resveratrol (RSV) can alleviate excessive liver triglyceride accumulation and reduce hepatotoxicity. In consequence our study aims to elucidate the role of RSV on the hypothetical improvement of palmitate-induced ER stress using a hepatic *in vitro* model. To do this, HepG2 cells were treated for 28 h with 25 μM RSV; 8h before the end of the treatment increasing concentrations of palmitate (PALM) were also added. Moreover, in other experiments, EPA (50 or 100 μM) or the LXR agonist T0901317 (10 μM) were added together with PALM. The obtained results indicate that some UPR effectors (XBP1-splicing, ATF4 and ATF6) were up-regulated concomitantly to several pro-apoptotic markers (CHOP and cleaved Caspase-3). On the other hand, ROS production was reduced when RSV was present in PALM-treated HepG2 cells. Interestingly, RSV decreases stearoyl-CoA desaturase 1 (SCD1) expression in HepG2, suggesting that the exacerbation of PALM effect in the presence of RSV could be linked to an increase in membrane saturation level. In this sense, both EPA and T0901317 reduced XBP1-splicing, CHOP expression and Caspase-3 cleavage in RSV + PALM-treated HepG2 cells, restoring in consequence the cellular homeostasis by membrane unsaturation enrichment. In conclusion, in the present study we have obtained an unexpected RSV effect, mediated by an increase in palmitate-induced ER stress and apoptosis; and we have shown that the RSV effect is partially mediated by SCD1 inhibition and membrane fluidity alteration (saturated/unsaturated ratio). Further research with primary hepatocytes is needed to elucidate whether this RSV effect in a 'saturated fatty acid context' is specific of transformed cell lines. In such case, this could be taken into account for a hypothetical adjuvant treatment for cancer therapy that will specifically target membrane fluidity of transformed cells through the 'RSV-forced' enrichment of membrane saturation and, in consequence, triggers ER-stress derived apoptotic signals alternative to classical mitochondrial apoptotic signals that could be altered in a transformed cell context (e.g. survivin).

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P12r-34
The N-terminal half of mammalian phosphofructokinase is sufficient to generate an active enzyme *in vivo*, but the C-terminal half is needed for stabilitiy as well as regulation

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Mammalian phosphofructokinase (PFK) evolved after duplication, fusion and divergence of an ancestral prokaryotic gene. It was suggested that the duplicated fructose 6-phosphate catalytic site in the C-terminal half became an allosteric site for the activator fructose 2,6-bisphosphate, and that both sites are formed by opposing N- and C-termini of subunits oriented antiparallel in a dimer [1]. However, it was recently reported that subunit association should instead involve N/N-C/C interaction, such that the N-terminus contains the active site and the C-terminus the allosteric site [2]. We present in this work that in fact the N-terminal halves face each other, forming by themselves an active enzyme *in vivo*, since expression of the N-terminal half of PFK-M was sufficient to restore growth in a PFK-deficient strain of *Saccharomyces cerevisiae*. The N-terminal half was, however, not stable *in vitro*. The C-terminus was not catalytic but was found to be necessary for stability of the enzyme, as co-expression of N- and C-termini generated a stable, fully active enzyme with physical, kinetic, and regulatory properties similar to those of wild-type tetrameric enzyme. This indicates that the separately translated domains can fold sufficiently well to bind to each other, that the interdomain interactions are strong enough to maintain the tetramer together, and that the alignment is sufficiently accurate and tight as to preserve metabolite binding sites and allosteric interactions. Interestingly, heterologous co-expression of *Dictyostelium discoideum* PFK and PFK-M termini, as well as chimaeric forms of these PFKs did not produce stable enzyme *in vitro*. Thus, the C-terminal domain appears to stabilize the whole enzyme only when in combination with the N-terminus of the same protein or from an evolutionary close isozyme, as shown previously for the chimaeric forms of mammalian M- and C-isozymes (3). MICINN (BFU2009-13114).

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P12-35
Quantitative analisys of the central metabolism regulatory interactions of *E. coli* in salt stress conditions

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Osmotic stress is a ubiquitous and well conserved mechanism designed to keep homeostasis that yields a multidimensional and complex response. The salt stress responses involve transcription, translation and post-translational changes that are reflected in changes in metabolite concentrations and fluxes. Due to its inherently complexity this phenomena can be well addressed through a systemic approach.

In this communication we deal with the adaptation of an *E. coli* culture medium to an increase of the osmolality of the medium. The responses, as determined by the changes in intracellular fluxes and intermediate concentrations, are mathematically modeled and quantified during the adaptation from a low to high osmolar medium. A set of special parameters, the osmotic kinetic orders, g_{OSn}, are defined; each one being associated with a given metabolic process.

The result of this work helps to understand the adaptive response to the osmotic stress in microorganisms. The knowledge obtained has a direct translation not only to unravel the network of internal signaling prompted by the medium salt concentrations but also has biotechnological applications.

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P12-36
The role of liver x receptor alpha in non alcoholic fatty liver disease

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Non Alcoholic Fatty Liver Disease (NAFLD) has become one of the leading causes of chronic liver disease in the worldwide. The spectrum of NAFLD ranges from various degrees of steatosis, inflammation and liver fibrosis. Liver fat accumulation has been suggested to be the first step of the pathogenesis of NAFLD. Numeros authors have demonstrated that LXRs (Liver × Receptors) may be key regulators of lipid metabolism and inflammatory signalling. LXRα is member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. LXRs may be involved actively in the pathogenesis of NASH and could be a potential target for therapy in NAFLD. The aim of this study was to evaluate LXRα mRNA expression in liver from morbidly obese women. We analyzed the livers of 143 Caucasian women: 6 lean (BMI < 25 kg/m^2) and 137 morbidly obese (BMI > 40). Among the morbid patients, we found 31 with normal liver, 26 with simple steatosis and 70 with steatohepatitis (NASH). Liver samples were obtained from morbidly obese women who underwent bariatric surgery by laparoscopic gastric by-pass. Those patients who had an acute illness, acute or chronic inflammatory or infective diseases, or end stage malignant disease, or who were taking medication that could alter lipidic or metabolic parameters, were excluded. LXRα mRNA levels were assessed by RT-PCR. Statistical analysis was performed using the IBM/SPSS statistical package (v.19.0). P Values < 0.05 were considered to be statistically significant. LXRα mRNA expression in liver was decreased in simple steatosis compared with controls and morbidly obese patients without significant liver disease ($P < 0.05$). In contrast, we were unable to find differences between morbidly obese with normal liver and NASH patients. Liver LXRα expression also correlated strongly with the steatosis. LXRα mRNA levels in liver are strongly related to the degree of steatosis in morbidly obese patients. In liver LXRα seems to be closely related to steatosis degrees but not with inflammation.

P12-37
Roles of the bone-derived osteocalcin as a GLP-1 secretagogue

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It has recently been reported that bone is an endocrine organ, since osteocalcin (OC) produced by osteoblasts is revealed to trigger the secretion of insulin; OC in uncarboxylated form (ucOC) but not carboxylated GlaOC acts as a potent insulin secretagogue, regulating energy metabolism by increasing energy expenditure and insulin sensitivity in the target organs. Incretin hormone glucagon-like peptide 1 (GLP-1) is a gastrointestinal hormone secreted by enteroendocrine L cells in the small intestine, and has a numerous physiological actions including potentiation of glucose stimulated insulin secretion, enhancement of β-cell growth and survival.

We here found that mouse small intestine and mouse enteroendocrine cell line STC-1 cells expressed Gprc6a, a putative ucOC receptor. Serum GLP-1 was increased by ucOC at both intraperitoneal and oral applications, but such an effect was not observed with GlaOC. STC-1 cells also responded to ucOC to secrete GLP-1 into the culture medium. GlaOC was effective only in the case of oral administration. Moreover, serum insulin level was increased in response to ucOC application, which was inhibited by GLP-1 receptor antagonist exendin(9-39), indicating that the insulin-releasing effect of ucOC is in part attributed to the action of GLP-1 released from the gut. These findings indicate that ucOC acts through its receptor in the small intestine, triggering secretions of GLP-1 and insulin.

P12-38
PPARα-dependent mechanism to maintain polyunsaturated fatty acid levels in the liver for whole-body fatty acid homeostasis

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Maintenace of fatty acid (FA) compositions in the membrane lipids is vital for cellular functions. De novo synthesis of fatty acids must be precisely regulated by reflecting the variable amounts and compositions of dietary FAs. The role of peroxisomal proliferator-activated receptor α (PPARα) in the mechanism to maintain FA homeostasis by balancing between the dietary FAs and *de novo* synthesis of FAs was investgated using wild type mice

and PPARα-null (KO) mice by feeding a standard laboratory diet or a fat-free diet. On a standard diet, PPARα-null mice show little abnormalities in lipid metabolism although expression of a key lipogenic enzyme SCD1 was quite low in KO mouse liver. However, compositions of major FAs in KO mouse liver were almost normal because of the compensation from the diet and/or adipose tissues where the expression level of SCD1 was maintained. In detail, the levels of unsaturated (u) FAs were higher in KO mouse liver, and it was found in our present study that the increase was caused by decreased expression of a key peroxisomal enzyme 2,4-dienoyl-CoA reductase (DECR2) in KO mouse liver and that the increased uFAs were the cause of repression of SCD1 through downregulaion of SREBP-1. RNAi knock-down of DECR2 in wild type mouse liver reproduced KO mice phenotype including downregulation of SCD1, and overexpression of DECR2 in KO mouse liver reproduced wild type phenotype. Furthermore, overexpression of SREBP-1 in KO mice recovered the expression level of SCD1 in the liver. On a fat-free diet, KO mouse liver robustly induced FA synthesis enzymes including SCD1 and resultant excess hepatic lipogenesis produced fat accumulations in adipose tissues. In contrast to wild type mouse liver, KO mouse liver expressed low level of a fatty acid transporter FATP1 and the reduced incorporation of essential uFAs from adipose tissues could not suppress excess lipogenesis. Thus PPARα plays an important role in whole-body lipid homeostasis by maintaining uFA levels in the liver through regulating expression of two key proteins, FATP1 and DECR2.

P12-39
Vasopressin stimulates the secretion of platelet derived growth factor A (PDGFA) in myofibroblasts and regulates the junctional permeability of colonic epithelial cells

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Studies *in vivo* demonstrate that vasopressin (AVP) stimulates proliferation of pericryptal myofibroblasts and decreases the crypt epithelial permeability by increasing the expression of junctional complex proteins. The aim of this study was to evaluate the effect of AVP (10 nM) on CCD-18Co myofibroblasts and the possible crosstalk of the CCD-18Co cells with the colonic epithelial T84 cell line. Cell proliferation was quantified by 5-Bromo-2′-deoxyuridine incorporation, the myofibroblast expression of EGF, PDGFA and PDGFB was measured by real-time PCR and the expression of junctional complex proteins by Western blot. AVP stimulated CCD-18Co proliferation and increased PDGFA expression ($p < 0.05$), and both effects were prevented when cells were pretreated by AVPR inhibitors. In addition, when myofibroblasts were treated with AVP and pre-incubated with the anti-PDGF antibody or anti-PDGF receptor inhibitor, hormonal effects were inhibited. Pretreatment of CCD-18Co cells with LY294002 or PD98059 (PI3K/AKT or Ras/Raf/MAPK inhibitors), blocked the AVP effects on cell proliferation. AVP had no direct effects on the expression of junctional proteins by the T84 cells; however, the expression of both β-catenin and claudin IV increased 25% when the T84 cells were incubated for 24 h with conditioned medium (CM) from CCD-18Co cells stimulated by AVP. The CM increased T84 proliferation ($p < 0.05$) and these effects were prevented by anti-PDGFA antibodies ($p < 0.05$). These results support the view that changes in colonic permeability during low-Na adaptation are mediated by the PDGFA secreted by myofibroblasts and that this occurs through the activation of the PI3K/AKT and Ras/Raf/MAPK pathways in response to raised AVP. Supported by BFU2006-08410, MEC, Spain

P12-40
Metal-binding characteristics of the transferrin/IGFBP-3 complexes

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Insulin-like growth factor-binding proteins (IGFBPs) bind IGFs, peptides with a broad range of metabolic and mitogenic activities. IGFBP-3 is the most abundant and it interacts with other biomolecules forming complexes that have IGF-dependent or independent functions. Transferrin (Tf) is one of these binding molecules. Tf is an iron-transporting protein that possesses two iron binding sites, whereas IGFBP-3 has one metal-biding domain. Both Tf and IGFs are important metabolic factors, so the formation of the Tf/IGFBP-3 complexes may influence iron metabolism and/or mitogenic/metabolic roles of the IGF system. The aim of this work was to investigate the characteristics of the Tf/IGFBP-3 complexes by the immobilized metal-affinity chromatography (IMAC) using different metal ions: Fe^{3+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+}, and sera from healthy individuals, persons with anemia and patients with colorectal carcinoma-induced anemia. Tf/IGFBP-3 complexes were isolated from the sera by double immunoprecipitation, using anti-Tf and anti-IGFBP-3 antibodies. Isolated complexes were analyzed by surface-enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS) employing IMAC/Cu^{2+} protein chip. Results have shown that the strongest interaction between Tf/IGFBP-3 complexes and the matrix occurred when Cu^{2+} was used. In SELDI-TOF MS complexes immobilized on IMAC/Cu^{2+} protein chip were ionized the least, whereas complexes bound to IMAC/Cu^{2+} column were eluted only with the EDTA solution. When iron was used as a metal chelator, complexes were completely eluted from IMAC column by its solution and the state of iron ionization (Fe^{3+} or Fe^{2+}) did not matter.

P12m-41
Existence of LPL pI isoforms in tissues from suckling rats: a comparative study with adults

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Lipoprotein lipase (LPL) is a glycoprotein enzyme with a key role in lipid metabolism. LPL hydrolyzes circulating triacylglycerides generating free fatty acids which are taken up by surrounding parenchimal cells. In adults, LPL is present in many tissues – including heart, muscle, adipose tissue, mammary gland- but not in liver. We have recently described the presence of at least, 8 pI isoforms in adult rat heart and post-heparin plasma (mature form of the enzyme) [1]. These pI isoforms might be due in part to post-translational modifications such as glycosilation. Years ago we studied the changes in LPL activity during post-natal development and we reported that the liver presents LPL activity until weaning [2]. Now we study the possible existence of LPL pI

isoforms in three tissues (heart, brown adipose tissue and liver) in suckling rats (15 days old). In order to compare the pattern of isoforms between different tissues at this developmental stage with adults, we have settled on the methodology to quantify the relative abundance (by densitometry) and the pI of each isoform. We have found that the offspring tissues studied present LPL isoforms with a pattern slightly different from that in adults. Surprisingly, offspring liver also presents pI isoforms of LPL, with a pattern more basic than that in the heart. The widespread existence of pI isoforms of LPL in different tissues and during postnatal development, suggests a functional involvement. These results lead us to study the molecular characterization of this heterogeneity and the possible impact on the regulation and function of these isoforms.

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P12-42
Indirect inactivation of tyrosinase in its action on 4-tert-butylphenol

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Under anaerobic conditions, the o-diphenol 4-tert-butylcatechol (TBC) irreversibly inactivates the met and deoxy enzymatic forms of tyrosinase. However, the monophenol 4-tert-butylphenol (TBF) protects the enzyme from this inactivation. Under aerobic conditions, the enzyme suffers suicide inactivation when it acts on TBC. This contribution proposes that suicide inactivation of the enzyme is not caused directly by the TBF but by the TBC accumulated in the reaction medium.

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[Correction after online publication 30 August 2012: Abstract text should not be the same as P12-48.]

P12-43
Hydroxylation of p-substituted phenols by tyrosinase. Further insight into the mechanism of tyrosinase activity

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A study of the monophenolase activity of tyrosinase by measuring the steady state rate with a group of *p*-substituted monophenols provides the following kinetic information: k_{cat} and the Michaelis constant, K_m. Analysis of these data taking into account chemical shifts of the carbon atom supporting the hydroxyl group (δ_1) and σ_p^+, enables a mechanism to be proposed for the transformation of monophenols into *o*-diphenols, in which the first step is a nucleophilic attack on the copper atom on the form E_{ox} (attack of the oxygen of the hydroxyl group of C-1 on the copper atom) followed by an electrophilic attack (attack of the peroxide group on the *ortho* position with respect to the hydroxyl group of the benzene ring, electrophilic aromatic substitution with a reaction constant ρ of −1.75). These steps show the same dependency on the electronic effect of the substituent groups in C-4. Furthermore, a study of a solvent deuterium isotope effect on the oxidation of monophenols by tyrosinase points to an appreciable isotopic effect. In a proton inventory study with a series of *p*-subtituted phenols, the representation of $k_{cat}^{f}{}_n/k_{cat}^{f}{}_0$ against n(atom fractions of deuterium), where $k_{cat}^{f}{}_n$ is the catalytic constant for a molar fraction of deuterium (n) and $k_{cat}^{f}{}_0$ is the corresponding kinetic parameter in a water solution, was linear for all substrates. These results indicate that only one of the proton transfer processes from the hydroxyl groups involved the catalytic cycle is responsible for the isotope effects. We suggest that this step is the proton transfer from the hydroxyl group of C-1 to the peroxide of the oxytyrosinase form. After the nucleophilic attack, the incorporation of the oxygen in the benzene ring occurs by means of an electrophilic aromatic substitution mechanism in which there is no isotopic effect.

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P12-44
Action of tyrosinase on ortho-substituted phenols: possible influence on browning and melanogenesis

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The action of tyrosinase on *ortho*-substituted monophenols – thymol, carvacrol, guaiacol, butylated hydroxyanisole, eugenol and isoeugenol – was studied. These monophenols inhibit melanogenesis because they act as alternative substrates to L-tyrosine and L-Dopa in the monophenolase and diphenolase activities, respectively, despite the steric hindrance on the part of the substituent in *ortho*position with respect to the hydroxyl group. We kinetically characterise the action of tyrosinase on these substrates and assess its possible effect on browning and melanognesis.

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P12-45
Identification and expression of *carS,* a central gene in the regulation of carotenogenesis in *Fusarium*

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Fusarium fujikuroi and *Fusarium oxysporum* are pathogenic fungi of rice and tomato, respectively. Both species have a rich secondary metabolism and share their ability to produce carotenoid pigments. We investigate the regulation of the genes of the carotenoid pathway, generically called car. The structural genes *carB, carRA, and carX*, needed to produce beta-carotene and retinal, are clustered and coregulated with carT, responsible for a late step of the pathway. Transcription of these genes is induced by light and derepressed in carotenoid-overproducing mutants, called *carS*. We have identified the regulatory *carS* gene in *F. oxysporum* thanks to the screening of T-DNA insertion mutants (collaboration with University of Amsterdam). We have confirmed the identity of *carS* by targeted gene disruption, and by subsequent complementation of the mutant phenotype. Moreover, the mutation of the orthologous gene in *F. fujikuroi* confirmed the same regulatory function for *carS* in this species, a conclusion further reinforced by complementation of the *carS* mutant of *F. oxysporum* with the *carS F. fujikuroi* counterpart.

CarS belongs to the RING finger family of regulatory proteins that includes CrgA, also involved in the regulation of carotenogenesis in the fungus *Mucor circinelloides*. We have investigated the regulation of the *Fusarium carS* gene. Light induces *carS* mRNA levels, but this stimulation was a 30-fold lower than that exhibited by structural genes of the pathway, such as *carB* or *carRA*. The*carS* induction by light was also detected in the *carS* mutants. Nitrogen starvation, a regulatory signal that controls the expression of many genes related with secondary metabolism in this fungus, upregulates transcriptionally carotenoid biosynthesis in *F. fujikuroi*. The mRNA levels of*carS* were modestly enhanced by nitrogen starvation. Experiments are in progress to elucidate the molecular mechanism by which the *CarS* protein exerts its regulatory function.

P12-46
Calcium signalling in mitochondria through malate-aspartate shuttle and calcium uniporter

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Calcium signaling to the mitochondria may take place through two different mechanisms. On the one hand, the classic pathway that involves calcium uptake through the calcium uniporter (MCU) and activation of three matrix enzymes (pyruvate, isocitrate and alpha-ketoglutarate dehydrogenases), that in turn will boost the TCA cycle and ATP production by the respiratory chain. On the other hand, extramitocondrial Ca^{2+} activation of the NADH malate-aspartate shuttle (MAS) through stimulation of the aspartate glutamate carriers (AGCs) will enhance the transfer rate of glycolytic redox equivalents to the mitochondria, and also boost respiration. This second mechanism takes place at cytosolic Ca^{2+} concentrations below the K_d of the MCU, does not require calcium entry to the mitochondria, and therefore, constitutes a novel and alternative mechanism to the classic one. In the brain, these two pathways are not independent, but linked through a shared substrate, alpha-ketoglutarate (alpha-KG): Upon calcium entry to brain or heart mitochondria, alpha-KG dehydrogenase increases its affinity for alpha-KG, that becomes limiting. This results in a reversible drop in alpha-KG efflux through the oxoglutarate carrier and in a drop in MAS activity. Because of their differences in cytosolic calcium concentration requirements, the MAS and Ca^{2+} uniporter-mitochondrial dehydrogenase pathways are probably sequentially activated during a Ca^{2+} transient, and the inhibition of MAS at the center of the transient may provide an explanation for part of the increase in lactate observed in the stimulated brain *in vivo*.

P12-47
Functional analyses of recombinant pufferfish growth hormone in lipid metabolism

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The main site of lipid storage differs among species in fish comprising two major groups. One group, which is represented by red seabream, accumulates lipid in their muscle and liver. The other group, including torafugu, does in their liver but hardly in muscle. We have shown in these fishes that the lipid content of liver and muscle is correlated with the mRNA level of lipoprotein lipase (LPL), a key enzyme in the incorporation of lipid from blood circulation system. In mammals, the expression of LPL is partially regulated by growth hormone (GH), via growth hormone receptor (GHR). The aim of the present study was to investigate the effects of recombinant GH on lipid metabolism in torafugu. Recombinant GH treatment on liver tissue slices decreased the mRNA level of LPL1 by 0.8-fold. On the other hand, the mRNA level of 14 kDa apolipoprotein, one of the components of serum lipoproteins related to lipid transport from liver to peripheral tissues, was increased by 1.5-fold after GH treatment. GH had no effect on the mRNA levels of other apolipoproteins, A-I and C-II. These results suggest that GH inhibits lipid uptake and promotes mobilization of lipid as lipoproteins in

torafugu liver, resulting in the reduction of its lipid content. Meanwhile, we also demonstrated that the hepatic mRNA level of GHR among various tissues was relatively lower in torafugu compared to other fish species, suggesting the weak GH/GHR signaling in torafugu liver. Hence, it is likely that GH/GHR signaling contributes to the liver-specific lipid accumulation in torafugu.

P12-48
Unraveling the effects of HMGA1 in the development of obesity and insulin resistance

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Adipocyte functionality is lost during obesity and has been related to impaired transcriptional regulation of the key factors that control adipogenesis. Among them, the high mobility group A1 (HMGA1) proteins may play an important role in adipogenesis. To examine the effects of adipose tissue HMGA1 expression in the development of obesity and insulin resistance, we generated transgenic mice overexpressing HMGA1 specifically in adipose tissue (aP2-HMGA1). HMGA1 expression in fat tissues was markedly increased compared with control animals. Intriguingly, HMGA1 transgenic mice showed decreased white (WAT) and brown (BAT) adipose tissue weight and lower triglyceride content than control mice. These transgenic mice were normoglycemic and normoinsulinemic and no differences in glucose tolerance and insulin sensitivity were observed between groups when fed a standard diet. In contrast, under a high-fat diet (HFD), body weight gain was lower in transgenic than in control mice. This was parallel to lower WAT and BAT weight. Transgenic mice also showed increased glucose tolerance and whole body insulin sensitivity and decreased levels of serum free fatty acid, triglyceride and glycerol compared to controls. Strikingly, the overexpression of HMGA1 in adipose tissue of transgenic mice led to a marked decrease in the expression of genes involved in fatty acid metabolism and in mitochondrial biogenesis and function, suggesting mitochondrial dysfunction of the adipose tissue in these transgenic mice. Currently, we are further analyzing the mechanisms by which HMGA1-mediated adipose tissue dysfunction in transgenic mice protects them against high-fat diet-induced insulin resistance and obesity.

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P12-49
Distribution of adipocyte in skeletal muscle of rainbow trout Oncorhynchus mykiss

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Energy metabolism in mammals is critically regulated by adipokines from adipocytes. Adiponectin is one of the adipokines which has been suggested to play an important role in the regulation of metabolism. Previous studies verified that rainbow trout adiponectin transcripts were abundant in muscle and marginally detected in adipose tissue. Heart fatty acid binding protein (H-FABP) is a member of intracellular fatty acid binding proteins. Also recently EST analysis of rainbow trout identified two adipose-specific genes, H-FABP and G0S2. However there is no detection of adiponectin transcripts in the other tissues. This research about adiponectin and H-FABP signaling pathway in fish will help in the understanding of energy metabolism and immunity regulation. We therefore produced adiponectin and H-FABP antibodies to investigate the distribution of adipocytes in rainbow trout skeletal muscle. We first evaluated the adiponectin antibody by Western blot analysis. The signal was detected around 75 000 on muscle. Since the molecular weight of fish adiponectin is around 25 000, we speculated that the 75 000 bands are trimmers. The results of H-FABP Western blot analyses, the signal was detected around 15 000. The rainbow trout H-FABP molecular weight are 14 000. We identified the signal detected around 15 000 as H-FABP. Subsequently, we conducted immunohistochemistry in muscle section (< 500 μm in thickness). Adiponectin signals were detected outside of muscle cells. H-FABP signals also showed similar distributions with adiponectin. Higher magnification revealed that adiponectin and H-FABP signals were observed in adipocyte-like cells in rainbow trout muscle tissues, quite different from mammalian cases. This paper will show the phylogenic analyses on adiponectin and H-FABP molecules and discuss about the evolutional development of physiological functions of vertebrate adipocytes.

P12-50
Endocrine FGFs, novel actors in the control of energy metabolism

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Fibroblast growth factor (FGF)15/19, FGF21 and FGF23 are members of the FGF family capable of exerting their effects as hormones, i.e. at distance from the secreting cell and tissue. Among them, FGF21 has been reported to exert powerful anti-diabetic actions. FGF21 lowers glucose levels and protects animal models from diet-induced obesity. FGF21 is considered to be mainly secreted by the liver, and white adipose tissue and pancreas were found to be major targets of FGF21 action. We found that FGF21 levels are strongly increased in mice just after birth, and this is due to the action of fatty acids coming from milk intake, which act on hepatic PPARalpha receptors thus inducing FGF21 gene transcription. We determined that brown adipose tissue is a main target of FGF21 action. *In vivo*, FGF21 promoted thermogenic activation of brown fat and, in brown adipocytes, FGF21 enhanced thermogenic gene expression, oxygen consumption and glucose oxidation. Moreover, brown fat appeared to be not only a target but also a source of FGF21 after thermogenic activation. Noradrenergic processes activate a PKA and p38 MAP-kinase-mediated induction of FGF21 gene transcription that leads to a massive output of FGF21 from brown fat into circulation. Obesity is associated with a paradoxical increase in blood FGF21 levels, and it has been claimed that obesity may be a ''FGF21-resistant'' state. Patients with HIV-associated lipodystrophy also showed enhanced levels of FGF21 in blood. We observed a strong repression in the expression of the FGF receptors mediating FGF21 effects and, specially, of beta-Klotho, an indispensable co-receptor required for FGF21 action in adipose tissue depots from obese and lipodystrophic patients. In contrast with FGF21, the levels in blood of FGF19, another endocrine FGF with beneficial effects on metabolism, were abnormally low. Reduced sensitivity to endo-

crine FGFs and low FGF19 are likely to contribute to systemic metabolic disturbances in obesity and lipodsytrophy.

P12-51
Metabolic compensation in Neurospora Circadian Clock

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CSP1 is a global transcription repressor of Neurospora. We show that CSP1 is controlled by the circadian clock and by glucose. The circadian period length decreases with increasing glucose concentration in csp1 mutant strains, while the period is compensated against glucose in a WT strain. Over-expression of CSP1 results in period lengthening and dampening of the clock rhythm. We show that CSP1 inhibits expression of the WCC by repressing the wc1 gene promoter. In a WT strain, glucose-dependent repression of wc1 transcription by CSP1 compensates for enhanced translation of WC1 at high glucose. This results in glucose independent expression of the WCC and hence in metabolic (glucose) compensation of the circadian period. Corresponding results were obtained with other carbon sources. Thus, the negative feed-back of CSP1 on WCC expression constitutes a molecular pathway that coordinates the interplay between energy metabolism and the circadian clock.

P12-52
Cooperation of adenosine with macrophage toll-4 receptor agonists leads to increased glycolytic flux through the enhanced expression of PFKFB3 gene

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Macrophages activated through Toll receptor triggering increase the expression of the A2A and A2B adenosine receptors. In this study, we show that adenosine receptor activation enhances LPS-induced *pfkfb3* expression, resulting in an increase of the key glycolytic allosteric regulator fructose 2,6-bisphosphate and the glycolytic flux. Using shRNA and differential expression of A2A and A2B receptors, we demonstrate that the A2A receptor mediates, in part, the induction of *pfkfb3* by LPS, whereas the A2B receptor, with lower adenosine affinity, cooperates when high adenosine levels are present. *pfkfb3* promoter sequence deletion analysis, site-directed mutagenesis, and inhibition by shRNAs demonstrated that HIF1 is a key transcription factor driving *pfkfb3* expression following macrophage activation by LPS, whereas synergic induction of *pfkfb3* expression observed with the A2 receptor agonists seems to depend on Sp1 activity. Furthermore, levels of phospho-AMP kinase also increase, arguing for increased PFKFB3 activity by phosphorylation in long term LPS-activated macrophages. Taken together, our results show that, in macrophages, endogenously generated adenosine cooperates with bacterial components to increase PFKFB3 isozyme activity, resulting in greater fructose 2,6-bisphosphate accumulation. This process enhances the glycolytic flux and favors ATP generation helping to develop and maintain the long term defensive and reparative functions of the macrophages.

P12-53
The relevance of aldolase A subcellular distribution in lung cancer cells

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Fructose 1,6-bisphosphate aldolase (ALDA) is a cytoplasmic enzyme involved in the formation of glycolytic complex. Except cytoplasm, the enzyme is also present in nuclei of squamous cell lung cancer cells. Searching for the role of aldolase in nuclei we found that the nuclear localization of the enzyme correlated with the proliferative activity of cultured cancer cells. A reduction of the rate of the cell's proliferation resulted in ALDA withdrawal from the nuclei. Moreover, a chemically-induced inhibition of the transcriptional activity of the cells also stimulated aldolase removal from the nuclei. These findings suggest that nuclear ALDA may participate in a regulation of the transcriptional activity.

To test the hypothesis that aldolase A is involved in the proliferation of cancer cells we investigated the effect of down-regulation of ALDA level on the proliferation of the cancer cells. We found that decreased amount of ALDA correlated with decreased rate of proliferation of KLN205 cells.

The results presented here demonstrates that nuclear ALDA may be involved in the regulation of proliferative activity of cancer cells presumably affecting the transcription of genes engaged in the progression of the cell cycle.

P12r-54
Effect of treatment with glucocorticoids FXR-mediated signaling pathway and bile acid homeostasis

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Long-term treatment with glucocorticoids (GC) has a wide range of adverse effects. Preliminary evidences suggest that GC induce changes in bile acid (BA) homeostasis. Here we have evaluated the effect of GC treatment on the FXR-mediated signalling pathway and on the expression of genes involved in the metabolism and enterohepatic circulation of BA. Rats were treated with dexamethasone, budesonide or prednisolone (50 mg/kg/i.p. of GC daily for 4 days). RT-QPCR and WB assays showed that dexamethasone induced hepatic downregulation of nuclear receptors (Fxr, GR and Shp), membrane transporters (Ntcp, Mrp4 and Bcrp) and enzymes (Cyp7a1 and Baat). In contrast, other ABC proteins, such as Bsep and Mrp2, the enzyme Cyp27a1 and

the nuclear receptor Lrh-1 were upregulated. HPLC-MS/MS analyses revealed increased serum levels of BA together with changes in the conjugation pattern. In HepG2 cells, dexamethasone and prednisolone treatment (20 μM for 24 h) induced downregulation of FXR, SHP, OSTβ and BCRP, whereas the expression of BSEP, CYP7A1 and BAAT were increased. The FXR agonist, GW4064 (1 μM for 24 h), induced downregulation of MRP2, BCRP, CYP7A1 and BAAT, whereas SHP, OSTα and OSTβ were upregulated. These effects of GW4064 were reduced by GC. When Alexander hepatoma cells were transfected with FXR a marked increase in BSEP expression was observed after treatment with GW4064 plus prednisolone. GW4064 alone caused upregulation of SHP and OSTβ. However, GC displayed antagonistic effects by reducing GW4064-induced SHP and OSTβ expression. In conclusion, GC reduce FXR/Fxr expression in the liver and alter its signalling pathway, mainly by modifying the expression of carriers and key enzymes involved in BA synthesis and conjugation, which results in changes in BA homeostasis.

P12r-55
Activation of glycogen synthase modulates its localization and interaction with RNA binding proteins in Sertoli cells

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Sertoli cells support the energetic demands of germ cells during spermatogenesis providing lactate from glucose as fuel, converting the 80% of the incorporated glucose into lactate and only 1-3% is used for lipids and glycogen synthesis. However, the expression of muscle glycogen synthase (MGS) does not correlate with levels of glycogen in Sertoli cells, suggesting another function for this enzyme. The aim of the study was to evaluate MGS protein interactions and localization in Sertoli cells to unveil putative functions. We explored its pattern in culture conditions, where MGS produce low levels of glycogen, and in induced culture conditions, where cells were treated with lithium or infected with adenoviruses encoding the Protein Targeting Glycogen (PTG), stimulating glycogen synthesis. In contrast to what has been described, lithium treatments did not increase glycogen amounts, but PTG strongly stimulated glycogen synthesis. Analyzing the MGS subcellular localization by confocal microscopy, we observed that MGS is distributed over the cytoplasm co-localizing with two RNA binding proteins (RBPs), Ago-2 and CPEB1, in non treated cells. In contrast, when cells where induced to activate MGS and forced to accumulate glycogen, the enzyme changed its localization pattern. In this condition, MGS is found in glycogen granules but not co-localizing with both RBPs. To corroborate this effect, we performed co-inmunoprecipitation assays. MGS co-inmunoprecipitated with Ago-2 and CPEB1 in cells presenting low levels of glycogen, but in lithium treated and PTG infected cells these interactions decreased. MGS interaction with RNA binding proteins highlights a putative role of this enzyme.Fondecyt-1110508, 1110571, Becas: CONICYT, Chile y MECESUP RM, Esc. Graduados y DID D-2011-09 UACh.

P12-56
A novel role for cAMP in the metabolism of Escherichia coli: regulation of the protein acetylation pathway

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Lysine acetylation is a well-established protein post-translational modification well conserved among all domains of life. In recent years, its relevance for metabolic regulation in prokaryotes and eukaryotes has been underscored. The regulation of flux partitioning at key bacterial metabolic nodes and regulatory proteins has been demonstrated [1,2]. In *Escherichia coli*, regulation of acetate metabolism by protein acetylation depends on a Gcn5-like protein acetyltransferase (*yfiQ*) and an NAD^+-dependent protein deacetylase (*cobB*).

We recently demonstrated that protein acetylation is linked to the metabolism of cells [3]. We have studied the environmental regulation of the expression of *cobB* and *yfiQ*. *cobB* is constitutively expressed from *nagK* promoter. The expression of *yfiQ* occurs from its own promoter and it is upregulated in the stationary phase and in the presence of non-PTS carbon sources. Positive regulation by cAMP-CRP was demonstrated; two putative CRP binding sites are necessary for full activity.

Gene deletion revealed that *cobB* is essential for growth on acetate, *yfiQ* deletion restoring growth of the *cobB* mutant. In addition, deacetylase activity is essential for metabolic efficiency, since its deletion decreases biomass yield due to excessive metabolic overflow. Enzyme assays and gene expression analysis demonstrate the inhibition of important acetate metabolism control points, such as acetyl-CoA synthetase or isocitrate lyase.

This PTM system contributes to fine tuning of several targeted enzyme activities, affecting homeostasis. Altogether, metabolic regulation by protein acetylation adds further complexity to our current picture of bacterial regulatory networks. Other physiological roles suggested, together with the existence of uncharacterized putative acetyltransferases are additional indicators of the relevance of this emerging topic.

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P12m-57
Discovery of lipoprotein lipase pI isoforms in humans

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Lipoprotein lipase (LPL) is a glycoprotein enzyme, with a central role in lipid metabolism, that hydrolyzes circulating triacylglycerols (TAG) into free fatty acids and glycerol. It is present in almost all tissues (the highest activities are found in adipose tissue, heart, and mammary gland). Its tissue-specific regulation, which is still poorly understood, directs the flow of circulating TAG in the body. Using proteomic techniques on rat heart and

post-heparin plasma (PHP), we previously demonstrated that LPL consists of a pattern of more than eight forms of the same apparent molecular weight but different isoelectric point (pI). Now we are investigating the possible existence of this LPL pI isoforms in humans. PHP was obtained from four healthy male volunteers through a collaboration with the 'Centre d'Investigació dels Medicaments' (CIM) – Sant Pau Hospital, Barcelona, SPAIN. Blood was collected 10 min after an intravenous administration of 50 UI of heparin/kg body weight and the LPL was prepurified and analyzed by bidimensional electrophoresis (2DE) and mass spectrometry techniques, as described in Casanovas A *et al* [1]. For the first time, we describe here that: (i) human mature LPL (from PHP) presents at least nine pI isoforms (pI between 6.8 and 8.6); (ii) these isoforms show a pattern which is similar in all volunteers and different to that of the rat.Now, we aim to study this heterogeneity in other human sources with high LPL activity such as white adipose tissue and milk, and the molecular origin of this pI heterogeneity. This research should help to uncover the role of LPL isoforms in the function and regulation of this central enzyme in intermediary metabolism.

Reference

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P12-58
Modulation of hepatic cholesterol metabolism during regeneration after partial hepatectomy

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Liver regeneration after partial hepatectomy (PH) is a very complex and coordinated compensatory hyperplasia produced by several stimuli that promotes proliferation in order to restore the liver mass and architecture. This process requires a well orchestrated network of growth promoting and inhibitory mediators to progress through the many phases of tissue regeneration. Modifications in metabolic pathways allow hepatocytes to maintain essential body functions. One of the main metabolic adaptations during liver regeneration affects to lipid constituents as not only a significant source of energy but also as important components for cell division. Although a link between liver regeneration after PH and transient steatosis has already been established, little is known about how hepatocytes manage cholesterol, an important structural component that modulates the permeability and fluidity of the plasma membrane contributing to the regulation of the activity of membrane proteins, cell trafficking and transmembrane signalling. Thus, we analyzed the modulation of hepatic cholesterol content, some enzymatic activities and gene expression of proteins involved in cholesterol metabolism during the first 72 hours after PH. We found that despite the already described increase in triglyceride (TG) content, free cholesterol (FC) diminished progressively reaching a minimum 48 hours after PH. The hepatic content in cholesteryl ester (CE) maintained stable during the 72 hours which made the ratio CE to FC increased. The decrease in FC was accompanied with a slight decrease in the gene expression of HMG-CoA reductase and the increase in that of apoA1. Regarding activities implicated in the FC-CE cycle, we found that acyl-CoA cholesterol acyltransferase activity diminished progressively while that of cholesteryl ester hidrolases do not change, probably in an attempt to maintain FC levels. As a conclusion, modulation of cholesterol metabolism supports the role of cholesterol in the liver mass restoring phase of liver regeneration after PH.

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P12-59
YebF (ClaR) – a novel positive regulator and its role in the regulation of the celB, ptcB, ptcA And bglS lactose and cellobiose assimilation genes in *Lactococcus lactis* IL1403

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The interesting feature of the *lactose*-plasmid-cured *Lactococcus. lactis* IL1403 strain is that it is still able to assimilate lactose but only after induction by cellobiose. In this process the cellobiose-specific PTS transport system, encompassing the PtcA, PtcB and CelB proteins, constituting EIIA, EIIB and EIIC PTS system components and a P-β-glucosidase, the BglS enzyme, are involved. This system is able to assimilate lactose due to the fact that cellobiose-specific proteins such as the CelB permease and P-β-glucosidase, BglS, have also an affinity for lactose. The transcription of individual genes of this system undergoes variable regulation, both as a result of the negative impact of the global regulator CcpA as well as activation by cellobiose. We propose that in this cellobiose-dependant activation of genes involved in cellobiose and lactose metabolism a hypothetical transcriptional regulator, namely YebF (now designated ClaR – cellobiose-lactose [*cel-lac*] DNA regions regulatory protein), could be involved. We have observed that the presence of cellobiose led to transcription activation of the *celB* and *bglS* genes, but not*ptcB* and *ptcA* genes. This phenomenon has not been observed when other sugars such as glucose, galactose or salicin have been used as carbon sources. In the current study we propose that this cellobiose inducible role is due to ClaR activity and discuss its probable mechanism of action.

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P12-60
Generation and analyses of metabolic disorder models in Drosophila

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The fruit fly, *Drosophila melanogaster* shares many genes with humans: approximately 75% of diseases-related genes in humans have orthologs in the fly, and it has been used as a model system to study molecular and genetic bases of human diseases. The models can be generated either by making loss-of-function mutants or transgenic flies bearing human disease genes. In the present study, we report several metabolic disorder models in *Drosophila*, which are defective in glycolysis, TCA cycle, fatty acid oxidation and the insulin signaling pathway. We performed metabolomics analysis using liquid chromatography mass spectrometry (LC-MS) to characterize the phenotypes of these models. We generated a Drosophila model of mitochondrial trifunctional protein (MTP) deficiency. Mitochondrial β-oxidation is the major metabolic system to catabolize fatty acids, producing acetyl-CoA by degrading fatty acids via a cascade of four reactions: dehydration, hydration, oxidation, and thiolysis. Mitochondrial trifunctional protein (MTP) catalyzes the latter three steps in the β-oxidation of long-chain fatty acids. MTP consists of a hetero-octamer of four MTPα and four MTPβ subunits. Both $Mtp\alpha^{KO}$ and $Mtp\beta^{KO}$ flies were viable, but had clear phenotypes, which include a shortened lifespan, defective locomotor activity, reduced fecundity, and abnormal lipid catabolism, which

was enhanced when animals were fasted. The phenotypes were generally more striking in $Mtp\alpha^{KO}$ than in $Mtp\beta^{KO}$ flies. We demonstrated that both $Mtp\alpha^{KO}$ and $Mtp\beta^{KO}$ flies accumulate acylcarnitine and hydroxyacylcarnitine, the intermediates of long-chain fatty acid metabolism, indicating that they have defects in long-chain fatty acid oxidation. These results suggest that the function of MTP is conserved between mammals and *Drosophila*, and that the *Drosophila* model should be useful to understand the molecular pathophysiology of MTP deficiency.

P12-61
Is glycogen overproduction a degenerative signal in the seminiferous tubule?

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High levels of energy are required to support all metabolic and morphological changes during spermatogenesis, the complex process by which the male germ-line stem cells (spermatogonial stem cells) self-renew and differentiate to produce spermatozoa. Glycogen is a highly branched glucose polymer. In some cases loss of glycogen homeostasis or alteration of the structural conformation of this polysaccharide may induce a pro-apoptotic signal. Previously our group demonstrated the expression of muscle glycogen synthase (MGS) in male germ epithelium of mice, this isoform being responsible for testicular glycogen synthesis. Using two transgenic models, a knockout (KO) of MGS and mice expressing an MGS form that is not regulated by phosphorylation (superactive-form), we evaluated the participation of glycogen in spermatogenesis. Both models were generated as nestin-CRE transgenics and they showed affected MGS expression in testes. The KO mice did not show morphological alteration in the germinal epithelium and exhibited normal spermatogenesis, evaluated by the presence of spermatozoa in the lumen of seminiferous tubules. In contrast, in the mice expressing the superactive enzyme, glycogen overproduction induced the degeneration of seminiferous tubules and an increase in apoptosis when compared with wild-type mice, as measured by immunodetection of cleaved caspase-3 and TUNEL assay. Testicular degeneration was estimated as a fibrotic signal by Masson′s tricromic stain and periodic acid-Schiff (PAS) staining confirmed the accumulation of glycogen. These findings highlight the role of GS activity and glycogen synthesis in testis development and spermatogenesis. These observations imply that any disregulation that involves impaired glycogen homeostasis could trigger the disruption of seminiferous tubules and cause infertility.

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P12-62
Cross-regulation during the simultaneous catabolism of aromatic compounds in *Pseudomonas putida* KT2440

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Aromatic compounds, some of which are major pollutants, are widely distributed in the environment and constitute a common carbon source for many bacteria. The aromatic catabolic pathways, as well as the regulation of the encoding genes, have been extensively studied in the laboratory. However, most studies rely on the use of a single aromatic compound and the catabolism of mixtures of such compounds, which is the common situation in nature, has received considerably less attention. *Pseudomonas putida* KT2440 is a model bacterium that is able to degrade a wide range of aromatic compounds under aerobic conditions following classical biodegradation pathways that form catecholic intermediates, e.g., gallate or 4-hydroxybenzoate (4HBA) that generates protocatechuate, or hybrid pathways that generate aryl-CoA intermediates, e.g., phenylacetate (PA) that generates phenylacetyl-CoA. Since cross-regulation between classical and hybrid aerobic pathways has not been studied so far in bacteria, we approach here this topic by using *P. putida* KT2440 growing in mixtures of PA and other aromatics such as gallate or 4HBA. By using *lacZ*-based gene fusion experiments we have shown that PA caused repression on the *Pb* and *Pt* promoters that drive the expression of the *gal* genes responsible of gallate degradation. Moreover, qRT-PCR gene expression studies of the *pobA* gene and biochemical analyses of the 4HBA monooxygenase (PobA)/protocatechuate dioxygenase (PcaGH) enzymes responsible of the first two steps of 4HBA degradation revealed that PA was also able to cause repression on the classical 4HBA/protocatechuate *ortho* catabolic pathway. To determine the molecular mechanism(s) underlying the PA-dependent response, we constructed a mini-Tn5 derived mutant genome library of *P. putida* KT2440, and we screened for strains lacking repression of the *Pt::lacZ* fusion when the cells were grown in gallate plus PA. Some of the mutants strains that did not show the PA-dependent repression of gal genes are being analysed, and the molecular basis of this unprecedented cross-regulation response will be discussed.

P12r-63
Antihypertensive effect of short-term treatment of grape seed procyanidin extract (GSPE). Contribution of nitric oxide and prostacyclins

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Metabolic syndrome (MS) is a combination of medical disorders such as hypertension, dyslipidemia and obesity that, when occurring together, increase the risk of developing cardiovascular disease and diabetes. Our group, have demonstrated that a grape seed procyanidin extract (GSPE) exhibit different beneficial properties on various parameters related to MS. The aim of this study was to evaluate the effects of GSPE on blood pressure (BP) and to study the potential antihypertensive mechanisms involved. Male spontaneously hypertensive rats (SHR) (17–20-week-old) were administered by gastric intubation water or 250, 375, and 500 mg/Kg GSPE (minimum n = 8 per group). Captopril (50 mg/Kg), a known antihypertensive drug, was used as a positive control (n = 8). Systolic and diastolic blood pressure (SBP and DBP) were recorded in the rats by the tail cuff method initially and 2, 4, 6, 8, 24, and 48 h post-administration. To determine the mechanisms involved, two groups of 15 animals were administered by intragastric gavage water or 375 mg/kg GSPE. Four hours post-administration, both groups were divided into 3 subgroups (n = 5), which were intraperitoneally administered

1 mL of saline, 30mg/kg Nw-nitro-L-arginine methyl ester (L-NAME), inhibitor of nitric oxide (NO) synthesis, or 5 mg/kg indomethacin, inhibitor of prostacyclin (PC) synthesis. SBP and DBP were recorded in the rats initially and 6 h after oral administration. GSPE produced a significant decrease dose-dependent of the SBP and DBP in SHR up to the dose of 375 mg/kg. Paradoxically, the dose of 500 mg/kg of GSPE caused the lowest effect. The maximum decrease of BP was observed 6 h post-administration and the initial values of BP were archived 48 h later. The 375 mg/kg dose produced an antihypertensive effect similar to Captopril. The antihypertensive effect of GSPE was completely and partially abolished in the rats treated with L-NAME and indomethacin, respectively. In conclusion, this study has demostrated the antihypertensive effect of the GSPE in SHR and this effect may be mainly mediated by NO and partially regulated by PC.

P12-64
The search of mitofusin 2 modulators identifies a link between Mfn2 and the novo synthesis of pyrimidines

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Mitofusin 2 (Mfn2) participates in mitochondrial fusion and regulates mitochondrial metabolism. Moreover, we have previously reported that Mfn2 is down regulated in muscle from obese or type 2 diabetic patients, and recently we have demonstrated that Mfn2 deficiency in liver or muscle leads to glucose intolerance and insulin resistance in mice. Therefore, activators of Mfn2 expression could be used as a valuable potential therapeutic strategy for the treatment of type II diabetes and obesity. For this purpose, we undertook a search for activators of Mfn2 expression by High Throughput Screening (HTS) using a FDA-approved library of 1120 compounds (Prestwick). HeLa cells stably expressing luciferase under the control of 2 kb of human Mfn2 promoter were incubated with the library and as a result Leflunomide was identified as a potent activator of Mfn2 transcriptional activity. Importantly, Leflunomide also increased Mfn2 mRNA and protein levels both in HeLa and C2C12 muscle cells, confirming Leflunomide as an activator of Mfn2 expression. Leflunomide decreases the synthesis of pyrimidines by inhibiting dihydroorotate dehydrogenase (DHODH). DHODH is an enzyme located in the inner mitochondrial membrane and links mitochondrial respiration with pyrimidine synthesis. Inhibitors of complex III, that also impair pyrimidine synthesis by indirectly inhibiting DHODH, produced an increase in Mfn2 expression in HeLa and C2C12 cells. The addition of external uridine, which reverses the deficiency in pyrimidines biosynthesis, reversed the increase in Mfn2 gene expression induced by Leflunomide or complex III inhibitors. These results suggest that Mfn2 upregultaion is triggered by the deficiency in pyrimidines and reveal a new unexpected link between pyrimidine biosynthesis and Mfn2 expression. Future studies will be performed to address the nature of this connection and its biological implications.

P12-65
Inherited metabolic disorders: a knockout mouse model of *Agl* Gene for the study of glycogen storage disease typelll

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Glycogen Storage Disease type III, a rare autosomal recessive disease of the metabolism of glycogen, is due to deficiency of amylo-1,6-glucosidase, 4-a-glucanotransferase enzyme (glycogen debranching enzyme); accumulation of glycogen occurs primarily in liver, heart and muscle, resulting in their progressive damage. Clinically, GSD III occurs with hepatomegaly, ketotic hypoglycemia with fasting, and elevated serum concentrations of transaminases, short stature, cardiomyopathy and myopathy. Skeletal myopathy manifest as weakness is not usually evident in childhood, but slowly progresses, becoming prominent in the third to fourth decade.

At present no treatment is available, and patients are administered with a high-protein diet and frequent feeds to maintain euglycemia. Here we present a constitutive knockout (KO) mouse model as the first animal model of GSDIII, created by deleting the three last exons which contain the glycogen binding site, and the glucosidase activity of *Agl* gene. Preliminary biochemical data confirmed the total absence of Agl enzyme in knockout animals and 50% in heterozygotes as far as muscle, liver and heart, while in the brain a minor expression was present even in the wild type. Since glycogen content in mouse muscle is 1/10 compared to human, dose and spectrum could not be determined. In adult KO liver glycogen resulted 8.3 g/100 g tissue (2.4 g in wild type liver), while the spectrum was in the normal range. Next steps will focus on histological analysis at different ages in order to monitor progressive glycogen storage, systematical dosage of the main biochemical parameters of glucidic metabolism, study of the metabolic transcriptional profile on the tissues mostly involved. Furthermore, physical exercise tests will be performed to ascertain and evaluate progressive muscle degeneration, as well as differential dietetic approaches.

P12-66
A mechanism for the substrate inhibition of pig kidney fructose-1,6-bisphosphatase

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Fructose-1,6-bisphosphatase, a key regulatory enzyme in gluconeogenesis, is a homotetramer which does not contain tryptophan. It has long been known that fructose-1,6-bisphosphatases isolated from different sources are inhibited by their substrate, but the mechanism of the inhibition has remained obscure. Three types of experiments were used to shed light on this: (i) fluorescence studies with mutants in which phenylalanine residues were replaced by tryptophan; (ii) exchange of subunits between wild-

type and Glu-tagged oligomers; (iii) kinetic measurements over a very wide range of substrate concentrations, subjected to detailed statistical analysis. At concentrations near the K_m value of 4 μM, the substrate Fru-1,6-P_2 caused an increase in the intrinsic fluorescence of the Phe219Trp enzyme whereas almost no perturbation was observed when the Phe232Trp enzyme was titrated. The transition was well described by a single binding isotherm, with a K_d of 7.9 ± 1.3 μM. The overall results indicate that two different classes of catalytic sites exist that differ greatly in their affinity for substrate. Binding of substrate to the low-affinity sites act as a ''stapler'' that prevents dissociation and hence exchange of subunits. The substrate inhibition results from the binding of fructose-1,6-bisphosphate to the low-affinity sites. (Acknowledgements: FONDECYT 1090740 (JCS); Fellowship MECESUP AUS0006 (JLA); DID-UACh).

P12-67
Longitudinal changes in adipo/cytokine levels after bariatric surgery: preoperative concentrations as predictors of weight reduction and insulin sensitivity recovery

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Bariatric surgery is an effective treatment for severe obesity. However, patients vary in terms of the achieved body weight loss, and the co-morbidities resolution such as type 2 diabetes. The use of the preoperative circulating levels of adipo/cytokines to predict weight loss and the amelioration of insulin resistance has not been performed yet. The aim of our study was to evaluate the performance of preoperative levels of different obesity related molecules as biomarkers for the prognosis of bariatric surgery.

We analyzed the adipo/cytokine circulating levels by ELISA in 90 Spanish women of European descent: 60 lean (BMI < 25Kg/m2) and 30 morbidly obese (MO) (BMI > 40). MO women were studied prior to laparoscopic bariatric surgery and at 6 and 12 months after surgery (AS). Statistical analysis was performed using the IBM-SPSS statistical package (v.19.0). Comparisons between groups were performed using t-Student's test. Multiple regression analysis was performed to analyse the predictive power of the different variables analysed. P values < 0.05 were considered to be statistically significant.

Adipokine levels (visfatin, chemerin, leptin) and pro-inflamatory molecules (TNF RI and RII, CRP) were significantly increased in MO and decreased AS. By contrast, HMW adiponectin and ghrelin levels were decreased in MO and increased AS. Bariatric surgery reduced BMI, fasting insulin and glucose concentrations at 6 and 12 months AS. The best predictor of the BMI reached after surgery was the preoperative values of BMI, but not the adipo/cytokine levels. In contrast, when HOMA2-IR at 6m AS was evaluated, the preoperative levels of TNF RII and visfatin predicted a 46% of its variability. In addition, visfatin was the molecule with higher predictive power for HOMA2-IR 12m AS. HOMA2-IR postoperatively was not predicted by basal HOMA2-IR or basal BMI in the cohort analysed.

In conclusion, the baseline BMI remains the best predictive factor for the achieved body weight loss AS. Interestingly, the pre-surgical visfatin concentrations independently predicted insulin sensitivity recovery.

P12-68
Introduction of L-aminoacid oxidase of Aspergillus niger (R3) by hydrogen peroxide

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We found L-aminoacid oxidase in peroxisome of Aspergilus niger R-3 fungus. There was no activity of the enzyme in homogenate. The enzyme is induced by addition of hydrogen peroxide to growth medium. The activity of the enzyme is seen not only in peroxisomal fractions, but also in homogenate. It correlates with results of many investigators, that low concentrations of hydrogen peroxide (0.1–50.0 mcM) activate different processes in the cells [respiratory burst in neutrophils and macrophages (Winn et al.; 1990), potassium channels (Kuo et al; 1993; Gamaley et al; 1994), prostaglandin E2 and I_2 secretion (Ager, Gordon; 1984), hydrolysis of phosphatidylinositol (Shasby et al; 1988).] Induced enzyme by H_2O_2 is stimulated by Fe^{2+}, Ca^{2+}, Cu^{2+}, ions, but inhibited by Zn^{2+} ion. These results are correlated with data of other researchers about transitory rise of intracellular Ca^{2+} ion when hydrogen peroxide was added to growth medium (Gamaley *et al,* 1994). Mechanism of action of hydrogen peroxide is discussed.

P12-69
ADCK2, a novel protein involved in coenzyme Q_{10} synthesis pathway

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The novel protein ADCK2 (aarF domain-containing protein kinase 2) has been predicted as an atypical mitochondrial kinase that is involved in the coenzyme Q_{10} synthesis pathway.

First we demonstrated its mitochondrial localization by subcellular fractionation techniques, specifically in the matrix and probably also in MAMs. We then analysed patients fibroblasts with typical symptoms of mitochondrial diseases, like muscle weakness and lactic acidosis, that carry mutations in *ADCK2* gene. We found in these cells low levels of coenzyme Q_{10} and a deregulation of other mitochondrial processes, like fatty acid β-oxidation or respiratory chain complex I activity. Silencing and overexpression of ADCK2 in several cell lines suggest a relationship between ADCK2 and the altered metabolic processes observed in patients fibroblasts. These results indicate that ADCK2 is involved in coenzyme Q_{10} synthesis pathway, affecting other metabolism processes in mitochondria.

P12-70
Carbohydrate metabolism gene expression changes in non-small cell lung cancer

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Lung cancer is one of the most frequent malignant tumor in human. It is well documented that most cancer cells metabolize glucose primarily via glycolysis, even in the presence of oxygen. The knowledge on the specificity of glycolytic metabolism of the cancer cells might provide new prognostic markers of lung tumor and its development. The purpose of this study was to investigate the expression of 14 glycolytic isozymes in the non-small cell lung cancer (NSCLC) during the carcinogenesis. Quantitative real-time PCR was performed using 65 tumor lung samples, of which a subset of 20 samples had available normal/tumor RNA pairs. The tissues were obtained from patients of Lower Silesian Center of Lung Diseases, Wroclaw, Poland. In almost all the cases, the predominant isozymes expressed the both in lung and tumor tissues were: enolase 1, phosphoglycerate mutase 1, platelet and liver form of phosphofructokinase (PFKP and PFKL), aldolase C and pyruvate kinase M2 (PKM2). The expression of regulatory enzymes of glycolytic pathway (PFKL, PFKM) was 3 to 4 times reduced in cancer samples, as compared to the lung tissue. The most statistically significant changes were observed for PFKL ($p < 0.0001$), PFKM ($p = 0.0011$), PKM1 ($p < 0.0001$) and aldolase A ($p = 0.0015$). Triose-phosphate isomerase (TPI) was the only enzyme which expression was up-regulated ($p = 0.0035$). This indicates that glycerol may play an important role as a metabolic substrate for NSCLC cells. Taking together, our findings suggest that in contrast to most other cancer cell lines, in NSCLC, glycolysis is not stimulated on the level of gene expression. However, the lack of over-expression of glycolytic enzymes does not exclude the existence of the Warburg effect in the NSCLC, since there is a possibility of the regulation of glycolytic pathway on the level of protein translation/degradation and/or posttranslational modifications.

P12-71
Looking at polyamine metabolism and functions: results from different experimental models of cardiovascular risk support their relation to body oxidant status

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Polyamines are small arginine-derived polycations involved in growth, differentiation, proliferation and cell death. In addition, several '*in vitro*' studies have demonstrated antioxidant properties for polyamines, mainly by quenching hydroxyl radical. However, the biological significance of such antioxidant functions has not been clearly established '*in vivo*'. In this way, we have evaluated the relation between the levels of main polyamines (putrescine, spermidine and spermine) in blood and/or tissues and the degree of oxidative stress under different experimental conditions. Studies were carried out in human and mouse models of cardiovascular risk, including diabetes and obesity. In all cases, polyamines were determined by HPLC. According to our results, (i) in alloxan-induced diabetic mice, spermine levels correlated with circulating malondialdehyde (MDA; a marker of oxidative stress) levels in almost all organs and tissues analyzed. Significant correlations with plasma MDA were also evident for spermidine and, to a lower extent, for putrescine. (ii) In type-2 diabetic patients, blood spermidine and spermine increased at the same time that plasma and urinary MDA. Intervention with oral arginine in these patients decreased both, MDA and polyamine levels. (iii) In obese children we observed an increase in both, blood polyamine levels and markers of oxidative stress. As polyamine levels were under strict metabolic control, our results support the idea that their increase may participate in the organic response to oxidative stress. In fact, several enzymes of polyamine metabolism (including ODC), have been described to be regulated at this level.

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P12-72
Regulation of transcriptional activity of PPARγ by lipin1

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PPARγ2 is a key transcription factor in adipogenesis. It is important to address how PPARγ acts as transcriptional activator in absence of precise ligands, maintaining the high expression levels of adipocyte-specific genes in adipocytes. Present study shows that lipin1 can activate PPARγ activity even in absence of ligands, by replacing the corepressors with coactivators like artificial PPARγ ligands, such as rosiglitazone. The activation domain in lipin1 resides in residue 217–399, where there is no conserved sequence and no homology with other lipin isoforms. This activation domain plays a critical role only in PPARγ activation. Activation domain of lipin1 is stimulated by p300. The physical interaction with PPARγ2 occurs at C-terminal region from residue 825 to 926. LXXIL motif in lipin1 is not important in interaction with PPARγ2 and its activation, while PPARα absolutely requires this motif for transcriptional activity.

P12-73
Relationship between renal distribution of PEPCK and glucocorticoid receptor in diabetic nephropathy

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Diabetic nephropathy (DN) is the commonest cause of end stage renal disease in Western world. Deterioration of renal function correlates with tubular alterations. One of these injurious mechanisms is enhanced renal gluconeogenesis, which occurs exclusively in the proximal tubule (PT) of the kidney. Phospho*enol*pyruvate carboxykinase (PEPCK) is a key enzyme in the gluconeogenic pathway, and it is mainly regulated at the transcriptional level. Glucocorticoids are considered the main positive modulators of PEPCK in kidney, but the exact distribution of the glucocorticoid receptor (GR) and PEPCK has not been reported. Here, we analyzed the expression of PEPCK and GR in normal and diabetic human and rat kidney. PEPCK mRNA and protein levels were enhanced in diabetic human and rat kidney, and its subcellular distribution was changed to compartments near the apical

membrane of PT. GR level was not changed in the diabetic kidney. In control rat kidney, GR was mainly detected in non-PT tubules and glomeruli, with nuclear staining in all of them. Not significant changes were appreciated in diabetes. In contrast, in non-diabetic human kidney GR was detected in different tubules but nuclear reaction was only revealed in PT. In diabetic patients, GR was mostly detected in PT, but no nuclear reaction was observed. Our data show that PEPCK is similarly regulated between rat and human kidney. Nevertheless, GR presents a distinctive expression between human and rat kidney which may indicate a diverse regulation and porpoises for its expression in different renal cells from these species. (FONDECYT 3120144; 1090694).

P13 – Molecular Bases of Pathology

P13-1
Transforming growth factor β1 C-509T gene polymorphism in atopic asthma

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Transforming growth factor beta-1 (TGF-β1) has both pro- and anti-inflammatory functions and may participate in the initiation, progression, and resolution of inflammatory and immune responses in the airways. Transforming growth factor beta-1 influences asthma by modulating allergic airway inflammation and airway remodeling. Polymorphisms in the regulatory regions of cytokine genes may affect the cytokine production and thus play a contributory role in the pathogenesis of asthma. Thus, the aim of this study was to investigate the association of TGF-β1 C-509T gene polymorphism with atopic asthma.

The TGF-β1 C-509T polymorphism was determined in 49 patients with asthma (24 atopic and 25 non-atopic) and 50 healthy controls using PCR-RFLP method.

Genotype and allele frequency distributions of TGF-β1 gene in patients did not show significant differences compared to controls. No differences in genotype frequencies between atopic and non-atopic asthma were observed. However, when CT and TT genotypes were grouped together and compared to CC genotype statistical significance was observed ($\chi^2 = 3.760$, $p = 0.05$). The presence of TGF-β1-509T allele ($\chi^2 = 4.196$, $p = 0.041$) was also significantly higher in atopic compared to non-atopic asthma. Our results indicate that the presence of polymorphic TGF-β1-509T allele is associated with asthma susceptibility in atopic subjects.

Keywords: bronchial asthma; atopic asthma; transforming growth factor β1; TGF-β1 C-509T polymorphism

P13-2
1α,25(OH)$_2$-Vitamin D3 and its analogue TX 527 inhibit the proliferation of Kaposi sarcoma cells through the modulation of NFκB pathway

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The Kaposi Sarcoma-associated herpes virus G protein-coupled receptor (vGPCR) is a key molecule in the pathogenesis of Kaposi Sarcoma (KS). Persistent expression and activity of vGPCR is required for tumor maintenance. It has been recently reported that Nuclear Factor κ B (NFκB) gene expression increased in experimental and human KS and that vGPCR potently activates the NFκB pathway. In the present work we have investigated whether 1α,25(OH)$_2$-Vitamin D$_3$ (1α,25(OH)$_2$D$_3$) and its less calcemic analogue TX 527 exert its growth inhibitory effects by modulation of the NFκB pathway in endothelial cells transformed by vGPCR (SVEC-vGPCR). Cell proliferation studies demonstrated that both 1α,25(OH)$_2$D$_3$ and TX 527, similarly to bortezomib, a proteosome inhibitor that suppressed the activation of NFκB, reduced both proliferation and NFκB activity of SVEC-vGPCR cells. Time-response studies showed that the hormone and its analogue significantly decreased NFκB and increased IκBα mRNA and protein levels. The increase of IκBα was accompanied by a reduction in p65/NFκB translocation to the nucleus. These responses were reversed when vitamin D receptor (VDR) expression was blocked by stable transfection of shRNA against VDR. In parallel with NFκB inhibition induced by TX 527, there was a down-regulation of inflammatory genes such as IL-6, MIP3α, and MCP. Altogether, these results suggest that the antiproliferative effects of 1α,25(OH)$_2$D$_3$ and its analogue TX 527 on endothelial cells transformed by vGPCR occurred by immune modulation of the NFκB pathway.

P13-3
Genetic and epigenetic analysis of *GRHL* genes in human skin cancers

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Grainyhead-like proteins (GRHL) belong to a highly conserved family of transcription factors that are critical for development and homeostasis of the surface ectoderm across a wide range of species. Based on literature data and our preliminary results we hypothesize that there is a link between *GRHL* genes and skin cancers. According to recent studies, the expression levels of *GRHL3* in human head and neck non-melanoma skin cancers are reduced by 90% in over half of the samples studied. We predict that this phenomenon will extend to other non-melanoma skin cancer types, as well as to melanoma, and that the alterations in *GRHL1* and *GRHL2* genes will also be found in these cancers. Our preliminary studies have shown that reduced level of *Grhl1* expression in mice increases the incidence of chemically induced (DMBA/TPA) non-melanoma skin cancers. Links to the process of carcinogenesis have also been shown for *GRHL2*.

The aim of the proposed research is to investigate whether various types of human skin cancers are accompanied by changes in the expression levels as well as genetic and epigenetic changes of *GRHL1-3* genes. We are going to specifically search for: upregulation or downregulation of the *GRHLs* expression, specific point mutations, loss of heterozygosity, copy number variation and changes of methylation profile in regulatory sequences. To detect and identify *GRHL* genes disruptions in skin cancers, we will use genetic and epigenetic approaches, such as New Generation Sequencing, DNA-methylation analysis and qRT-PCR.

Our findings will provide new molecular insights into the links between the *GRHL* genes and epidermal neoplasia, which will enhance our knowledge of the molecular basis of skin carcinogenesis in the human context.

P13-4
Myotonia congenita: novel mutations in CLCN1 gene and functional characterizations in Italian patients

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Myotonia congenita is an autosomal dominantly or recessively inherited muscle disorder causing impaired muscle relaxation and muscle weakness, abnormal currents linked to the chloride channel gene (*CLCN1*) encoding skeletal muscle ClC-1. We describe 12 novel mutations: p.Val536Leu, p.Gly845Ser, p.Gln812X, p.E500X, p.Arg338X, c.2403 + 1G > A, p.Val947-Glu, p.Thr533Ile, c.1110delC, p.Ile197Arg, c.2276insA Fs800X, p.Trp164Arg in 22 unrelated Italian patients. Of selected missense mutations (p.Trp164Arg, p.Ile197Arg, p.Gly845Ser, p.Gly190Ser) were characterized the biophysical properties in tsA cell model. In the physiological range of muscle membrane potential, all the tested mutations, excepting p.Gly845Ser, reduced the open probability, increased the fast and slow components of deactivation and affected pore properties. This suggests a decrease in macroscopic chloride currents impairing membrane potential repolarization and causes hyperexcitability in muscle membranes. Detailed clinical features are given of the 8 patients characterized by cell electrophysiology. Since nonsense mutations were not analyzed by whole-cell patch clamp, and we meant to verify the expression of these variants at RNA level, RealTime-PCR was performed on selected nonsense (p.Arg338X and p.Gln812X), and on the missense variants functionally studied. Results confirmed a decreased expression of nonsense compared to wild type and missense mRNAs, with the exception of W164R showing the lowest transcript amount. Further analysis are needed to clarify these findings. These data expand the spectrum of *CLCN1* mutations and contribute to genotype-phenotype correlations. Furthermore, we provide insights into ClC-1 protein structure and its physiological role in the maintenance of membrane resting potential.

P13r-5
HIF-1 and c-Src mediate increased glucose uptake induced by endothelin-1 and connexin43 in astrocytes

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In previous work we showed that endothelin-1 increases the rate of glucose uptake in astrocytes, an important aspect of brain function since glucose taken up by astrocytes is used to supply the neurons with metabolic substrates. In the present work we sought to identify the signalling pathway responsible for this process in primary culture of rat astrocytes. Our results show that endothelin-1 promoted an increase in the transcription factor hypoxia-inducible factor-1α (HIF-1α) in astrocytes, as shown in other cell Furthermore, HIF-1α-siRNA experiments revealed that HIF-1α participates .types in the effects of endothelin-1 on glucose uptake and on the expression of GLUT-1, GLUT-3, type I and type II hexokinase. We previously reported that these effects of endothelin-1 are mediated by connexin43, the major gap junction protein in astrocytes. Indeed, our results show that silencing connexin43 increased HIF-1α and reduced the effect of endothelin-1 on HIF-1α, indicating that the effect of endothelin-1 on HIF-1α is mediated by connexin43. The activity of oncogenes such as c-Src can up-regulate HIF-1α. Since connexin43 interacts with c-Src, we investigated the participation of c-Src. Interestingly, both the treatment with endothelin-1 and with connexin43-siRNA increased c-Src activity. In addition, when c-Src activity was inhibited neither endothelin-1 nor silencing connexin43 were able to up-regulate HIF-1α. In conclusion, our results suggest that endothelin-1 by down-regulating connexin43 activates c-Src, which in turn increases HIF-1α leading to the up-regulation of the machinery required to take up glucose in astrocytes. Connexin43 expression can be reduced in response not only to endothelin-1 but also to various physiological and pathological stimuli. This study contributes to the identification of the signalling pathway evoked after connexin43 down-regulation that results in increased glucose uptake in astrocytes. Interestingly, this is the first evidence linking Cx43 to HIF-1, which is a master regulator of glucose metabolism.

P13-6
Electrophoretic analysis of plasma/serum proteome of rats exposed to satic electric field

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The external Static Electric Field (SEF) of man-made origin brings to the substantially increased SEF background in a human environment the biological activity of which is a moot question. The paper reports on rats' blood plasma/serum proteome modifications by means of 1D polyacrilamide gel electrophoresis and clotting process alterations after the short and long term SEF exposures of 200 kV/m. The results indicate decrease of fast alfa1 and alfa2 globular proteins in plasma coinciding with clotting acceleration after the short term SEF, and attenuation of clotting dependent proteome modifications reflected with incomplete coagulation after the long term SEF exposure. Increased lysozyme activity in serum unlike plasma was observed after both SEF exposures. Applied model of the high-voltage SEF environment indicates dependence of biological systems' functioning on the external SEF.

P13-7
Analysis of ZEB2 expression in stromal cells of pancreatic ductal adenocarcinoma(PDA), pancreatitis and normal pancreas

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Introduction: Most carcinomas are composed not only of genetically modified (mutant cancerous) epithelial cells, but also contain significant amounts of genetically normal (activated stromal) cells of the microenvironment. Stromal cells present in tumors at all stages of carcinogenesis. In the case of PDA stromal cells can occupy up to 80% of the tumor mass. One possible mechanism of formation of such stromal component of the tumors of the pancreas is epithelial-mesenchymal transition (EMT).

The purpose of our work was to analyze gene expression of ZEB2 gene-one of the possible regulators of EMT in stromal cells of PDA. For this purpose, a comparative study of ZEB2 expression in cultured stromal cells from tissues of normal pancreas, pancreatitis and PDA, was made. In epithelial tumor cell lines it was studied the effect of overexpression of ZEB2 on the promoter activity of genes important for tumor progression in tumor cells of pancreas.

Results and Conclusion: It was found differences in localization of ZEB2 protein in primary stromal cells of PDA compared with normal. In our experiments by western analysis it was shown an increased expression of ZEB2 protein in stromal cells of the tumor and pancreatitis, compared with normal cells. Using the method of co-transfection by reporter promoter constructions and by plasmid, encoding the full length cDNA of ZEB2, it was shown that increased expression of the gene ZEB2 leads to reduced activity of the genes E-cadherin, a-SMA and increased activity of the genes vimentin and survivin. Activity of these genes is important for tumor progression. These results allow considering ZEB2 as a specific marker of stromal cells of the pancreatic cancer. The work was supported by grant 11-04-12069 of the Russian Foundation for Basic Research.

P13-8
Activation-induced cytidine deaminase promotes oncogenesis of ultraviolet light-independent epidermal cancer from keratinocytic and melanocytic origin

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Most skin cancer develops after ultraviolet (UV) light-induced DNA damage and repair errors. However, significant portion of skin cancer appears to occur independently from UV light because squamous cell carcinoma and malignant melanoma, two major types of skin cancer, often occur in light-protected areas such as oropharyngeal mucosa and footpad. Such UV-independent epidermal cancer is speculated to have a causal link with chronic inflammation. However, how chronic inflammation leads to cancer is unclear. We propose here that activation-induced cytidine deaminase (AID) is a mechanistic link between chronic inflammation and skin cancer. AID is an enzyme essential for DNA cleavage involved in immunoglobulin class switch and somatic hypermutation. We found that human primary cultured keratinocytes and melanocytes, and also squamous cell carcinoma (SCC) and melanoma cell line expresses AID after stimulation with LPS, poly(I:C), 12-O-tetradecanoylphorbol 13-acetate (TPA), or inflammatory cytokines such as TGF-β and TNF-α. In a mouse skin cancer model using 7,12-dimethylbenz[a]anthracene (DMBA) and TPA, tumor frequencies were elevated by transgenic expression of AID from keratin 14 promoter, and decreased by genetic deletion of AID. Overexpression of AID in human keratinocyte cell line caused point mutations in *TP53* gene. These results suggest that AID is a DNA damaging factor involved in inflammation-associated epidermal cancers.

P13-9
Cell death induced by inhibition of glucose metabolism: role of Bcl-2 proteins and autophagy

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Alterations in glucose utilization or availability are associated with a number of pathologies including cancer, diabetes, ischemic stroke and myocardial infarction. It is not understood why some cells, and in particular, tumor cells, are highly sensitive to cell death induced by glucose deprivation. We are studying the mechanism by which cells die when glucose metabolism is inhibited in two different manners: by employing 2-deoxyglucose (a non-metabolizable glucose analog) or depriving cells of glucose. In rhabdomyosarcoma cell lines, glucose deprivation induces necrosis that cannot be inhibited by necrostatin-1, while 2-deoxyglucose induces Noxa-mediated mitochondrial apoptosis. Addition of different carbon/energy sources to 2-deoxyglucose-treated cells indicated that apoptosis is not associated with loss of ATP but rather with endoplasmic reticulum stress and the eIF2-alpha-ATF4 pathway. 2-DG promoted loss of the antiapoptotic protein Mcl-1, probably due to general inhibition of translation, since both eIF2-alpha phosphorylation and inactivation of the mTOR pathway were observed.

All these events (ER stress, energetic stress and inhibition of the mTOR pathway) are known to induce autophagy. Indeed, 2-DG induces autophagy, and inhibition of autophagy at different levels sensitizes cells to 2-DG. Surprisingly, this was not the case when cells were challenged by depriving them of glucose. We will present data regarding the role of autophagy in necrotic or apoptotic cell death induced by 2-DG or glucose deprivation.

P13-10
Cyclooxygenase-2 is a target of microRNA-16 in human hepatoma cells: role in apoptosis

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COX-2 expression has been detected in human hepatoma cell lines and in human hepatocellular carcinoma (HCC); however the contribution of COX-2 to the development of HCC remains controversial. COX-2 expression is higher in the non-tumoral tissue and inversely correlates with the differentiation grade of the tumor. It has been demonstrated that COX-2 expression depends on different cellular pathways involving both transcriptional and post-transcriptional regulation. The aim of this work was to assess whether COX-2 could be regulated by microRNAs in human hepatoma cell lines and in human HCC specimens since these molecules contribute to the regulation of genes implicated in cell growth and differentiation. Our results show that miR-16 silences COX-2 expression in hepatoma cells by two mechanisms: a) by binding directly to the microRNA response element (MRE) in the COX-2 3'-UTR and promoting translational suppression of COX-2 mRNA and b) by decreasing the levels of the RNA-binding protein HuR. Moreover, a reduced miR-16 expression correlated to high levels of COX-2 protein in liver from patients affected by HCC. As a consequence of COX-2 downregulation by miR-16, an increased apoptosis was found in human hepa-

toma cell lines. Our data suggest an important role for miR-16 as a post-transcriptional regulator of COX-2 in HCC and implicate the potential therapeutical application of miR-16 in those HCC with a high COX-2 expression.

P13-11
Difference in adhesion capacity of mutated antibiotic resistant strains of *Haemophilus influenzae* in the A549 and Hec 1B carcinoma cell lines in the presence of cloranfenicol

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Haemophilus influenzae is a gram negative naturally transformable coco bacillus that colonizes the human nasopharynx. A specific strain of *H. influenzae* – 07 – has a modified membrane protein that provides antibiotic resistance to cloranfenicol and tetracycline. The genome sequence of the protein was obtained based in the sequence published in the Gene Bank (GI: 2981106). The primers were designed based on this sequence and after a PCR reaction we obtained the amplicon for the porin gene. This amplicon was used in a transformation technique on the selected strains of *H. influenzae* – 13, 46,47 C and Beta-lac – respectively, to obtain the mutants. This gene transference provided an antibiotic resistance to cloranfenicol in the selected strains. The mutated strains and the wild strains were tested for cell adhesion in Hec 1B and A549 cell lines in a RPMI medium with and without the selected antibiotic (the citotoxicity for the antibiotic was also acquired and the cells lines were viable). The cell lines were chosen because of the infection patterns of H. influenzae. The bacteria usually causes pneumonia and otitis media. Thus, we selected the A549 that is a human lung adenocarcinoma epithelial cell line carcinoma and has special properties such as surfactant production. And the Hec 1B that is a human endometrial adenocarcinoma. The test was performed by verifying if the adhesion rates were augmented with the use of the chosen antibiotic – cloranfenicol – in both wild and mutant strains. The wild strains showed very low levels of adhesion with and without the antibiotic in the RPMI medium for the Hec 1B cells. On the other hand the mutants were 10% more adherent in Hec1B cell lines. Furthermore these mutants also had a slightly different adhesion rate when presented with the antibiotic, augmenting it. In the A549 cell line the adherence rates were similar for wild and mutant strains. And when present with antibiotic on the medium a small difference was observed. We can conclude that the antibiotic resistance was really acquired and the gene probably more expressed in order to present a better survival rate for the bacteria. Distinctive patterns of adhesion in different cell lines can mean that the bacteria have more interaction with the cell. Further studies will be conduct to verify its difference.

P13-12
Analysis of the expression and the translocation to the host cell of SlrP, an effector of the *Salmonella* type III secretion systems

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Salmonella is able to inject several proteins, called effectors, into the eukaryotic host cell via two distinct type III secretion systems (T3SS). Effectors manipulate the host by interfering with eukaryotic signal transduction pathways. SlrP is a *Salmonella* effector with ubiquitin ligase activity that interacts with human thioredoxin and the endoplasmic reticulum chaperone ERdj3. This effector can be secreted through T3SS1 and T3SS2, two *Salmonella* secretion system with very different patterns of expression. We studied the expression of *slrP* at the transcriptional level, using a *lacZ* fusion, and at the protein level, using a 3xFLAG fusion, in different environmental conditions. Some of the relevant factors affecting *slrP* transcription were aeration, pH, magnesium concentration, and presence of butyrate. A screen to find genetic factors controlling *slrP* expression identified the two-component system PhoP/PhoQ, the phosphorelay system RcsC/RcsD/RcsB, and the T3SS1 regulators HilD and HilA. Finally, the minimal signal necessary for translocation of SlrP into epithelial cells and macrophages was investigated using fusions with the catalytic domain of CyaA, a calmodulin-dependent adenylate cyclase from *Bordetella pertussis*.

P13r-13
YobG regulates *steA* through the two-component regulatory system PhoPQ in *Salmonella enterica*

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Salmonella enterica produces several diseases, including acute gastroenteritis, bacteremia and typhoid fever. To manage to do this, it uses type III secretion systems (T3SS) for translocation of bacterial proteins to host cells. These proteins, called effectors, interfere with host signal transduction pathways, making possible the bacterial uptake, and the survival and proliferation of bacteria inside vacuoles. *S. enterica* possesses two T3SS encoded in two pathogenicity islands called SPI-1 and SPI-2, respectively.

SteA (*Salmonella* translocated effector A) is one of the few proteins that are secreted by both of the *Salmonella* T3SS. Once inside the host cell, SteA is located in the Golgi apparatus, but its genetics and biochemistry are unknown. We carried out a screen to look for transcriptional regulators of *steA* and we found YobG as a candidate. The expression of this membrane peptide is activated by PhoPQ, a two-component regulatory system that controls the expression of a large number of genes that mediate adaptation to low $Mg2+$ environments and/or virulence in several Gram-negative species including *Salmonella enterica* and *Escherichia coli*. YobG also acts as a feedback inhibitor of the PhoPQ system. In fact, we have found that the putative main regulator of *steA* is PhoP, the response regulator of the PhoPQ system, and that there is a putative PhoP box in the promoter region of *steA*. We are studying the regulation of *steA* by PhoPQ and the role of YobG as a conditional regulator of this two-component regulatory system during the infection by *Salmonella*.

P13r-14
Nbs1 is required for macrophage normal proliferation and activity

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease that is caused by mutations in *NBS1*. NBS1 is a member of the MRN complex which has an important role in DNA damage repair and signaling. Individuals with NBS are characterized by microcephaly, growth retardation, increase tumor rate and

also immunodeficiency. Macrophages have a crucial role in both innate and adaptive immunity. They can be proinflammatory when activated to fight infection and clear pathogens and alternatively activated to repair damage caused to surrounding tissues. We hypothesized that the immunodeficiency in these patients is due in part to a diminished ability of macrophages to deal with DNA stress when NBS1 is not functional. We have found that NBS1 is differently expressed in macrophages stimulated with LPS, IFNγ, M-CSF and IL-4. This regulation is independent of transcription and dependent on proteosome activity. Moreover we have compared bone marrow derived macrophages from WT and *Nbs1*$^{B/B}$ mice that lack the Nbs1 N-terminus and have similar cellular phenotypes as NBS patients. Macrophages mutant for Nbs1 proliferate less and have defects in progressing to S phase of the cell cycle. These macrophages also have higher of NOS2 that would impair their proper function. Our results provide new insight in the role of NBS1 in macrophages and the potential importance of macrophages in NBS immunodeficiency.

P13m-15
Sustained JNK activation in pancreatic β cells impairs insulin signaling and autocrine function but it is not sufficient to induce cell death

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It has been described that JNK (c-Jun N-Terminal Kinase) activity is abnormally increased in several tissues in conditions of insulin resistance and type 2 diabetes. For instance, both the hyperglycemia-induced production and release of IL-1B and the excess of saturated fatty acids related to obesity, lead to the activation of JNK in insulin-producing cells causing pancreatic B-cell dysfunction. In addition, it has been shown that JNK inhibition by chemical inhibitors prevents IL-1B-induced apoptosis of the pancreatic islets.

To achieve *in vivo* activation of JNK in pancreatic B cells, a transgenic mouse was generated expressing a constitutively active form of the JNK MAP2K (Mitogen Activated Protein 2 Kinase) MKK7D (MAPK Kinase 7D) in B-cells. Despite these mice have no morphostructural affectation of the pancreatic islets or differences in the total insulin content, they have defective glucose homeostasis showing glucose intolerance and decreased insulin secretion in response to hyperglycemia. This reduction in glucose-induced insulin release is B-cell autonomous, as it is reproduced in isolated islets, and JNK activity dependent, as it is reverted by a specific inhibitor of JNK.

At molecular level, B-cells with activated JNK have a blockage in the insulin signaling pathway that reduces the secretion of insulin and the expression of insulin target genes. The treatment with rosiglitazone, an insulin-sensitizing drug of the thiazolidinedione family that inhibits JNK activation, restored insulin secretion in response to glucose in isolated islets and *in vivo*. All these data indicate that the activation of JNK is sufficient to promote central insulin resistance but is not sufficient to induce islet hyperplasia or B-cell death.

P13-16
Implications of the Hereditary Hemorrogic Telangiectasia (Rendu-Osler-Weber Disease) in the immune system. Are HHT patients immunocompromised?

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal vascular dominant disorder characterized by recurrent epistaxis, cutaneous telangiectasies and visceral arteriovenous malformations (AVMs) that affect many organs. Mutations in ENG or ALK-1, two members of the TGF-β signaling pathway, account for more than 90% of HHT clinical cases. Several case reports indicate that HHT patients have a putative higher susceptibility to infectious complications as cerebral abscesses, septicemia and osteomielitis among others, but their immune function have been poorly studied. To deep into the implications of HHT in the immune system, we have analyzed human samples from HHT patients and generated a murine model. Here we have identified for the first time the expression of endoglin on murine peritoneal macrophages and analyzed the *in vivo* induction during the differentiation from monocytes to macrophages. In humans, the infectious incidence of HHT was studied in a Spanish HHT cohort, from the national HHT reference center. In addition, isolated blood mononuclear cells from patients present a differential gene expression. The functional analysis of differentially expressed genes revealed that several immune responses were affected as cytokine production, which was confirmed by *in vitro* assays after LPS stimulation. To highlight the possible involvement of HHT in abnormalities of the immune system, we have generated an endoglin knock-out mouse specific of the myeloid lineage. Recent data obtained on myeloid endoglin KO mice, will confirm that endoglin is involved in the regulation of the immune response. A greater knowledge about factors contributing to the development of life-threatening and mild infections in HHT, would improve patient information, diagnosis and prevention.

P13-17
Deciphering the role of autophagy in Dictyostelium discoideum during *Mycobacterium marinum* infection

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The social amoeba *Dictyostelium discoideum* is a macrophage surrogate that can be used to study host-pathogen interactions with *Mycobacterium marinum*, a close related species of *Mycobacterium tuberculosis*. Recent evidence suggests that the autophagy pathway is implicated in the clearance of intracelullar bacteria upon infection in other model organisms, but the molecular mechanisms of this process are not fully understood. Therefore, we have developped tools to study autophagy in *D. discoideum* in order to further investigate its role upon *M. marinum* infection. First, we have monitored and analyzed autophagy by the apparition of fluorescent autophagosomes, using the early and late autophagy markers Atg18 and Atg8. Besides, we have observed and characterized the induction of autophagy upon treatment with the drug AR-12 by fluorescence confocal microscopy and transmission electron microscopy. Moreover, we have arrested

the flux of autophagy by the addition of NH_4Cl and confirmed the already reported progressive accumulation of autolysosomes with the genetic construct RFP-GFP Atg8. Preliminar results show an increase of autophagy related structures in *M. marinum* infected cells. Finally, we have noticed an unexpected colocalization of Atg18 to mycobacteria since early times post infection, which suggests new roles and dynamics for the autophagy pathway during the infection process.

P13-18
A CyaA-based screen reveals that PipB2 can be translocated through the Salmonella pathogenicity island 1-encoded type III secretion system

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Salmonella enterica possesses two type III secretion systems, T3SS1 and T3SS2, that are encoded by *Salmonella* pathogenicity island 1 (SPI1) and 2 (SPI2), respectively, and exhibit different patterns of expression. Through these systems, bacteria are able to inject proteins, called effectors, into the eukaryotic host cell cytoplasm. The effectors interfere with some signal transduction pathways. Whereas T3SS1 is important for invasion, T3SS2 is necessary for the intracellular part of the infection. There is a great interest in the identification of effectors and in the characterization of their roles in the interaction between bacterial pathogens and host cells. We carried out a screen to detect T3SS effectors based in the generation of chromosomal fusions with the catalytic domain of CyaA from *Bordetella pertussis*. This is a calmodulin-dependent adenylate cyclase. Since calmodulin is present in eukaryotic cells but not in bacteria, translocation of the fusion protein to the host cells can be detected as an increase in the concentration of cAMP in the infected cell culture. We detected several *Salmonella* effectors under conditions that favored T3SS1 expression. Surprisingly, one of the effectors detected was PipB2, previously described as a T3SS2 that interacts with the motor protein kinesin-1 and participates in the positioning of *Salmonella* containing vacuoles. Translocation of PipB2 through T3SS1 was confirmed in human epithelial cells (HeLa), murine macrophages (RAW264.7) and rat fibroblasts (NRK). Analysis of the kinetics of translocation into RAW264.7 cells showed that PipB2 is translocated through T3SS1 at the beginning of the infection (30 min-2 h) and through T3SS2 at longer infection times (8–16 h). Translocation into HeLa cells was T3SS1-dependent at short and long time points post-infection. We also found that the amino-terminal 10 amino acids were able to direct a significant level of translocation of the heterologous protein CyaA. Our results suggest that PipB2 could have additional roles in the initial stages of infection.

P13r-19
SrfJ is a *Salmonella type* III secretion system effector whose synthesis is induced by *myo*-inositol

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Salmonella enterica is a facultative intracellular Gram-negative bacterium responsible for gastroenteritis and systemic infections in many animals including humans. *Salmonella* uses two virulence-related type III secretion systems, T3SS1 and T3SS2, to translocate proteins, called effectors, from the bacterium into the eukaryotic host cell. The genes encoding the structural components of T3SS2 and some of its effectors are located in *Salmonella* pathogenicity island 2 (SPI2), and their expression is positively regulated by SsrB. *srfJ* (SsrB regulated factor J) is a gene located outside SPI2 that is regulated by SsrB. We studied the expression of *srfJ* in different media and we found that *srfJ* is expressed under SPI2 inducing conditions. pH and Mg^{2+} concentration are relevant factors in the regulation of this gene. Analysis of secretion to the culture medium and of translocation into murine macrophages suggested that SrfJ is, in fact, a substrate of the T3SS2. The N-terminal 20-amino acid segment of SrfJ was enough to direct translocation of a heterologous protein through the secretion system. Finally, we identified PhoP as a positive regulator, and RcsB and IolR as negative regulators of *srfJ*. IolR is the repressor of genes involved in *myo*-inositol utilization. Our experiments suggested that although SrfJ is not necessary for *myo*-inositol utilization, it is strongly induced in the presence of this sugar.

P13r-20
Differentially expressed proteins and phosphoproteins of the pancreas during the early phase of cerulein-induced acute pancreatitis. A 2-DE analysis

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It is reasonable to assume that the development of acute pancreatitis (AP), an inflammatory disease of the pancreas, would be based on early events whose unmasking would be important for the study of AP at the molecular level. Proteomic analysis of tissue and subcellular fraction extracts might reveal new potential diagnostic or prognostic biomarkers. AP was induced by two s.c. injections of 20 μg cerulein/kg body weight at hourly intervals. Comparative 2-DE analyses (using different pH gradients) of rat pancreas, and different pancreatic subcellular fraction (to simplify the protein maps) extracts obtained 2 hours after the first injection of cerulein (early phase of AP) were performed. The differentially expressed proteins were identified by MALDI-TOF-TOF MS analysis. Changes in some altered proteins were further assessed by Western blotting. To date, we have conclusively identified 24 proteins or protein fragments expressed differentially between diseased and control pancreas. To our knowledge, some of the up-regulated proteins have never been described in AP: (i) serpins B6 and A3L, serine protease inhibitors; (ii) thioredoxin-like 1, a cell redox homeostasis protein; and (iii) STRAP, a TGF-β signal transduction pathway element. STRAP up-regulation is especially interesting because it acts as a negative regulator of ASK1-mediated JNK/p38 signaling cascade, the activation of MAP kinases being one of the key pathogenic mechanisms in pancreatitis and other inflammatory diseases. Since a functional link between the ASK1 and TGF-β signaling pathways remains to be investigated, STRAP might be a good candidate. We are currently performing the last series of IEF, 2-DE separation, and image analysis of the phosphoprotein maps that were revealed by fluorescence with the Pro-Q Diamond Reagent. Preliminary results point to differences in the maps, and the proteins involved are now being identified.

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P13-21
Isoenzymatic expression of Na,K-ATPase in islets of Langerhans from rats with normal and impaired glucose tolerance

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Na,K-ATPase is ubiquitously expressed in all eukaryotic cells where it acts as a regulator of Na+ and K+ homeostasis. The Na,K-ATPase pump consists of (αβ)2. The α-subunit contains the catalytic and ligand binding sites. The β-subunit is a glycosylated protein, and its role in Na,K-ATPase enzyme function remains somewhat obscure. Four α- and three β-isoforms have been described in mammals. The isoenzymatic expression pattern is tissue- or cell line-specific and is a factor accounting for its regulation. Isoenzymatic distribution in islets of Langerhans is unknown however previous work has shown altered Na,K-ATPase activity in pancreatic β-cells from glucose intolerant subjects and alterations in isoenzymatic expression in other tissues has been described in type 2 diabetes. The aim of this work was to determine isoenzymatic expression of Na,K-ATPase in the islets of Langerhans from rats with normal and impaired glucose tolerance. Pancreata from control (Wistar) and glucose intolerant rats (GIR) were excised and fixed and prepared for immunohistochemistry analysis of α1, α2, α3 and β2 isoforms. Isoforms α1, α2, α3 and β2 were found in islets of Langerhans suggesting that the isoenzymes α1β, α2β and α3β of Na,K-ATPase are expressed. The isoenzymes α1β and α3β were found throughout the whole islet whereas $\alpha_2\beta$ isoenzymes were predominantly expressed at the periphery. Compared to controls, an increased expression of isoenzymes α2β and a decreased expression of isoenzymes α1β of Na,K-ATPase was found in GIR islets of Langerhans. In conclusion, since isoenzymatic expression may contribute to the differential sensitivity of Na,K-ATPase to substrates, these alterations may contribute to abnormal regulation of Na,K-ATPase activity in pancreatic β-cells from GIR.

P13-22
Macrophage-mediated natural clearance of Amyloid A is not impaired in the absence of immunoglobulins or central complement factors

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AA amyloid deposits regress upon the reduction of SAA concentration and this proves that AA amyloid can be naturally cleared. Here, we have used genetically modified mouse strains to investigate the importance of immunoglobulins and complement components, common constituents of AA deposits, on the natural clearance of amyloid. AA amyloidosis was induced in wild-type, B-cell deficient ($J^{H-/-}$) and complement factor three and four deficient ($C3C4^{-/-}$) mice. The resolution of amyloid was studied after staining with amyloid-specific dyes (Congo Red and pFTAA) in spleen and liver collected between 2 and 20 weeks post-induction. Resolution of spleen amyloid started in wild-type and $C3C4^{-/-}$ animals 4 weeks after discontinuation of the inflammatory stimuli and only after 9 weeks in $J^{H-/-}$ mice. Amyloid clearance subsequently intensified, and approximately 40% of the original amyloid mass remained at time point 20 weeks post-induction. A much faster clearance was observed in liver and 13 weeks post-induction only traces of amyloid surrounding central veins were detected. The amyloid removal was accompanied, in both spleen and liver, by an up-regulation of CD68, a phagosome lysosome marker, on the infiltrated F4/80+ macrophages, and at later time points these cells contained intracellular amyloid. Western blot analysis of amyloid with AA/SAA specific antibodies showed that amyloid resolution occurred through a gradual cleavage of AA fragments. In newly formed amyloid, we identified four distinct AA proteins with molecular sizes between 6 and 11 kDa, and the 11 kDa fragment was the predominant finding. In contrast, at 20 weeks post-induction, the remaining amyloid was predominantly composed of ~6 kDa AA fragments. Interestingly, this material showed partial resistance to protease K treatment. In conclusion, natural clearance of amyloid progressed similarly in wild-type, agammaglobulinemic and complement-deficient mice. This unexpected finding suggests the existence of a powerful innate-immune system mechanism for amyloid removal.

P13-23
CALM3 promoter methylation associates with genes down-regulation in patients with colorectal cancer

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Colorectal cancer (CRC) is one of the most common human malignancy worldwide. The disease arises through a variety of chromosomal and genetic abnormalities, as well as epigenetic alterations. The aberrant methylation of CpG islands in the promoter gene region is an epigenetic event that can cause gene silencing.

In this study we first used micro-array technology to perform expression profiling of 16 CRC specimens. One of the genes found to be significantly under-expressed in 11 CRC samples comparing to matching, healthy colon mucosa was calmodulin-3 (*CALM3*). We further confirmed *CALM3* down-regulation by quantitative PCR. Tumour had on average 1.7 times less *CALM3* than healthy tissue ($p < 0.003$). To establish if DNA promoter methylation correlate with down-regulation of this gene we performed a methylation-specific high-resolution melting profiling of *CALM3* promoter region on a larger group of CRC samples. A methylated CpG site was found in a near proximity to transcription start site in 5′ upstream region. Hypermethylation occurred in 7 CRC specimens with down-regulated *CALM3*, but not in any other out of additional 27 examined bisulfite DNAs. DNA methylation and *CALM3* down-regulation correlated with upper colon cancer location rather than rectal.

CALM3 codes for calmodulin protein, which is a ubiquitous, highly conserved calcium sensor protein involved in the regulation of a wide variety of cellular events including motility and cell cycle progression. Our study demonstrates that *CALM3* promoter methylation might be a possible mechanism causing down-regulation of this gene in substantial proportion of CRCs.

P13-24
Bioinformatics approach to protein-protein interactions of WT1 isoforms

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Analysis of protein-protein interactions involved in pathogenesis of Acute Myeloid Leukemia (AML) is important from the point of understanding underlying mechanisms, but also for the development of chemicals for its treatment. Wilms' Tumor 1 (WT1), a transcription factor alternatively spliced in two sites resulting in four isoforms (+)/(−) 17aa and (+)/(−) KTS, is overexpressed in the majority of AML cases. Although 41 proteins are described as protein partners of WT1, there is not enough information about their preferences towards specific isoforms. Since the long-range properties of proteins govern their interactions, we employed the Informational Spectrum Method, a bioinformatics tool for analysis of long-range protein-protein interactions for examination of WT1 isoforms and described WT1 interactors.

Analysis of informational characteristics of WT1 isoforms showed substantial differences between (+) and (−) 17aa isoforms, and not so pronounced differences between (+) and (−) KTS isoforms. Six proteins were shown to considerably differ in their interactions with (+) and (−) 17aa isoforms: Par-4, UBC9, BMZF2, Cited2, HtrA2 and ZNF224. The process with highest variability between WT1 isoforms was shown to be sumoylation and similar difference between (+) and (−) 17aa isoforms was noticed for both UBC9 and SUMO. Isoform-specificity of sumoylation was unique compared to ubiquitination – interactions with UBCH8 and ubiquitin were same for all WT1 isoforms.

WT1 (+) 17aa isoforms are preferentially expressed in AML and they are probably involved in its pathogenesis. Our study pointed to Par-4 and HtrA2, proteins implicated in apoptosis, as potential isoform-specific interactors of WT1 and targets for treatment development. Also, sumoylation is underlined as an isoform-specific process and its potential involvement in AML should be further investigated.

P13-25
Exendin-4 ameliroates renal injury by decreasing inflammation and ROS production in diabetic mice

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Aims: Exendin-4, glucagon like peptide-1 receptor agonist, has been using treatment of type 2 diabetes due to its blood glucose lowering properties. Kidney is one of the major organs affected by hyperglycemia in diabetic patients. In this study, we aimed to investigate the curative effects and possible mechanism of exendin-4 on kidney injury in streptozotocin induced diabetic mouse.
Methods: BALB/c male mice were divided into four groups for this investigation. The first group was given citrate buffer only, the second group was administered exendin-4 alone, the third group received STZ, and the fourth group was given both STZ and exendin-4. Exendin-4 (3 μg/kg) was treated by daily subcutaneous injection for 30 days after the animals were rendered diabetic by administration of STZ (200 mg/kg). Renal histology, proliferating cell nuclear antigen (PCNA) positivity in tubular cells, renal lactate dehydrogenase (LDH) activity, reactive oxygen species (ROS) production and IL-1β protein level in kidney tissues were evaluated.
Results: Exendin-4 decreased degenerative morphological changes seen in diabetic kidney. Besides, PCNA positive tubular cells were also significantly decreased by administration of exendin-4. In addition, increased LDH activity and ROS levels observed in diabetic kidney were markedly regressed with exendin-4 treatment. Moreover, exendin-4 supressed the level of IL-1β, an inflamatory cytokine, in diabetic mouse kidney.
Conclusion: These results suggest that exendin-4 may decrease renal injury seen in streptozotocin-induced diabetic mice by decreasing inflammation and ROS production.

P13-26
Synthesis of multivalent pseudo-mannosides as inhibitors of Mannose Binding Lectine, a potential target in human stroke

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It has been demonstrated that recombinant human complement C1-inhibitor (rhC1-INH) has a potent protective effect in cerebral ischemia/reperfusion injury by inhibiting activation of complement lectin pathway. This biological effect is due to the binding to mannose binding lectin (MBL) [1], one of the recognition molecules of the lectin complement pathway, probably by its mannose-enriched N-terminal domain. It has been previously demonstrated that linear pseudodi- and pseudotrisaccharides are adequate ligands for lectins that recognize mannose (i.e. DC-SIGN) and that multivalent carbohydrate systems interact efficiently with receptors and compete with the natural ligands [2].We show that multivalent presentations of glycomimetics based on dendrons produce very potent inhibitors of MBL and lead to neuroprotection. We synthesized new mannosylated mimetic molecules to evaluate the relevance of the lectin pathway in reperfusion ischemic injury. Newly synthesised compounds have been characterised for their binding to MBL by surface plasmon resonance (SPR) and selected one has been assessed for its ability to prevent ischemic damage in mice. The compound was administered intravenously to ischemic mice and neurological deficits and infarct volume were evaluated.Polyman002, a dendron exposing four copies of the pseudo-trisaccharide bind MBL with a $K_D = 2.3 \pm 0.7$ μM and induced a significant reduction of neurological deficits and ischemic volume *in vivo*.Our findings, together with those recently published by Cervera [3], indicate that MBL inhibition may represent a novel therapeutic target for stroke.

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P13-27
Serotonin-containing mast cell granules are rich in polyphosphate and similar to acidocalcisomes

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Polyphosphate (polyP) is a proinflammatory agent and a potent modulator of the human blood-clotting system. The presence of polyP of 60 phosphate units was identified in rat basophilic leukemia (RBL-2H3) mast cells using specific enzymatic assays, ureapolyacrylamide gel electrophoresis of cells extracts, and staining of cells with 4,6- diamidino-2-phenylindole (DAPI), and the polyP-binding domain of Escherichia coli exopolyphosphatase (PPX). PolyP co-localizes with serotonin- but not with histamine-containing granules. PolyP levels greatly decreased in mast cells stimulated to degranulate by IgE. Mast cell granules were isolated and found to be acidic and decrease their polyP content upon alkalinization. In agreement with these results, when RBL-2H3 mast cells were loaded with the fluorescent calcium indicator fura-2 acetoxymethyl ester to measure their intracellular Ca2+ concentration ([Ca2+]i), they were shown to possess a significant amount of Ca2+ stored in an acidic compartment different from lysosomes. PolyP derived from RBL-2H3 mast cells stimulated bradykinin formation and it was also detected in human basophils. All these characteristics of mast cell granules, together with their known elemental composition, and high density, are similar to those of acidocalcisomes. The results suggest that mast cells polyP could be an important mediator of their proinflammatory and procoagulant activities. Inorganic polyphosphate (polyP) is a linear anionic polymer of orthophosphate (Pi) residues linked by high-energy phosphoanhydride bonds present in all cells. In both prokaryotes and eukaryotes, polyP is packaged, along with calcium and other cations, in acidic organelles termed acidocalcisomes. The discovery of acidocalcisome-like organelles in acidocalcisomes have been conserved over evolutionary time or have appeared more than one time by convergent evolution. PolyP is secreted from platelets upon activation and has considerable procoagulant and anti-fibrinolytic activitie. Recent studies have demonstrated that polyP acts at four points in the bloodclotting cascade. PolyP of the size range that accumulates in bacteria and other microorganisms (long-chain polyP) potently triggers the contact pathway, accelerates Factor V activation, and enhances fibrin polymerization. In contrast, short-chain polyP polymers, of the size secreted by human platelets, are less potent than long-chain polyP in triggering contact activation or in enhancing fibrin polymerization, while retaining full ability to promote Factor V activation. Both short- and long-chain polymers potently stimulate the activation of Factor XI by thrombin as well as Factor XI autoactivation. PolyP initiate the contact pathway by activating Factor XII to Factor XIIa, leading to thrombin and fibrin generation via the intrinsic pathway of coagulation and also to bradykinin formation by kallikrein-mediated high molecular weight kininogen cleavage. Bradykinin is the ligand of kinin B2 receptor, which activates various intracellular signaling pathways that lead to inflammatory reactions. Like acidocalcisomes, human platelet dense granules are spherical, acidic, electron dense, and have high concentrations of cations and polyP. Platelet dense granules and acidocalcisomes also share the system for targeting of their membrane proteins via adaptor protein-3 (AP-3) complex. Both organelles are considered lysosome-related organelles (LROs), defined as cell type-specific modifications of the post-Golgi endomembrane system that have a variety of functions and share some characteristics with lysosomes. LROs include melanosomes, lytic granules in lymphocytes, platelet dense-granules, basophil and mast cell granules, neutrophil azurophil granules and others. Mast cell granules are also similar to acidocalcisomes. They are spherical, electrondense, have high concentrations of cations (calcium, magnesium, potassium, sodium, and zinc) and phosphorus and, as acidocalcisomes, have been proposed to possess a proton pump (vacuolar ATPase) and a calcium pump (Ca2+-Mg2+-ATPase) (Chakravarty and Nielsen, 1980) to maintain their acidity and high calcium levels, respectively. Mast cells and blood basophils are associated with pathological conditions such as asthma, allergy and anaphylaxis. Basophils are a population of circulating white blood cells that are functionally similar to mast cells. Both cell types share many common features, such as high-affinity IgE receptor expression, metachromatic staining, and histamine release, among others. Mast cells also have key roles in the modulation of inflammation and the enhancement of adaptive immune responses, where they contribute to pathogen clearance and regulation of angiogenesis. The most striking feature of mast cells is that their cytoplasm is filled with dense metachromatic granules. When activated, mast cells secrete their cytoplasmic granules to the media, releasing a wide selection of cell modulators such as heparin, histamine, serotonin, neutral proteases, growth factors, and proinflammatory cytokines. These secretory granules are distributed in different subsets wherein histamine and serotonin are localized separately. Interestingly, mast cell lysates have been shown to have a procoagulant effect, and chronic urticaria, which is an autoimmune disease associated with the presence of histamine releasing autoantibodies against the high affinity IgE receptor of mast cells and basophils, is associated with activation of blood coagulation. Furthermore, mast cell-derived heparin, as polyP, is also known to initiate the contact pathway-mediated bradykinin formation. The similarities between acidocalcisomes and mast cell granules and the roles of mast cells in inflammation and hemostasis suggested to us that polyP may be present in these organelles. In this report, we demonstrate that, as acidocalcisomes, mast cell granules are rich in polyP. PolyP co-localizes with serotonin- but not with histaminecontaining granules. Mast cell polyP greatly decreased when cells were stimulated to degranulate by IgE and was able to stimulate bradykinin formation, suggesting that mast cell polyP is an important mediator of mast cell proinflammatory and procoagulant activities.

P13-28
Ca^{++} influx and tyrosine kinases trigger adenylate cyclase toxin endocytosis. Activation of a repair-response in target cells

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Humans infected with *Bordetella pertussis*, the whooping cough bacterium, show evidence of impaired host defenses. This pathogenic bacterium produces a unique adenylyl cyclase toxin (ACT) which enters human phagocytes and catalyzes the unregulated formation of cAMP hampering important bactericidal functions of these immune cells that eventually, likely in synergy with its haemolytic capacity, causes cell death by apoptosis and/or necro-

sis. In a previous work we demonstrated that ACT is internalized into macrophages together with other membrane components, such as the toxin receptor, the integrin CD11b/CD18 (CR3), and GM1. The toxin-triggered internalisation occurs in these cells through two different routes of entry, a chlorpromazine-sensitive clathrin-mediated endocytosis and a clathrin-independent internalisation. An intracellular vesicular localization of ACT has also been observed in T cells by other authors. The goal of this study was to determine whether ACT uptake may take place into cells devoid of receptor. We present a detailed study addressing the endocytosis of ACT in three different cell lines: CHO cells, CHO-CR3 cells, in which the CR3 integrin has been stably transfected, and J774A.1 macrophages, in which the intrinsic expression of CR3 has been knocked down. We show that ACT is rapidly eliminated from the cell membrane either in CR3-positive as negative cells by a calcium-mediated internalization. Activation of Src Tyr kinases and caveolin phosphorylation induced by ACT are other features of the endocytic strategies triggered. In addition, we find that the particular entry pathway followed by the toxin into the different cell lines might depend on the target cell physiology. Remarkably, we show for first time that cells injured by ACT are able to restore their membrane, suggesting that a repair response might be triggered by the target cell to protect it from the toxin injury.

P13-29
Molecular mechanisms of ADAM17 activation – new directions from a functional genomic screen

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Ectodomain shedding plays several roles in cellular homeostasis, regulating cell growth, adhesion, migration and many other important cellular functions. This proteolytic event is mainly executed by A Disintegrin And Metalloproteases (ADAMs) and can be induced by different stimuli, such as activation of PKC by the phorbol ester PMA. However, the molecular mechanisms are poorly understood. In order to understand the regulatory machinery of ADAM17 activation, a whole-genome siRNA screen was performed, using ADAM17-mediated shedding of Heparin Binding-Epidermal Growth Factor-like Growth Factor (HB-EGF) as a model system. The assay utilized HT1080 cells over-expressing alkaline phosphatase (AP)-tagged HB-EGF. Treating the cells with PMA results in a rapid release of active HB-EGF from the cell surface, which can be quantified by immunofluorescent staining of AP on the non-permeabilized adherent cell layer or by measuring the AP enzymatic activity in the cell supernatant. Stringent selection criteria resulted in 81 hits, encouragingly including both ADAM17 and PKCα, which is a known regulator of ADAM17. Validation of knockdown efficiency is currently ongoing, and the most promising hits are being tested for cell-type and substrate specificity. Hits of priority are being taken for further mechanistic studies. ADAMs are involved in both cancer and neurodegeneration and are thus potential drug targets. Identifying regulators of ADAM protease activity may allow more precise protease intervention by preventing interaction with these regulators – an exciting alternative to traditional protease inhibition that may afford a distinct pattern of specificity.

P13r-30
Role of HERG1 potassium channel in both malignant transformation and disease progression in head and neck squamous cell carcinomas

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Evidence indicates that HERG1 voltage-gated potassium channels could represent new valuable membrane therapeutic targets and diagnostic/prognostic biomarkers in various cancers. This study is the first to investigate the expression pattern of HERG1 potassium channel subunit in both primary tumors and precancerous lesions to establish its clinical and biological role during the development and progression of head and neck squamous cell carcinomas (HNSCC). HERG1 protein expression was evaluated by immunohistochemistry in paraffin-embedded tissue specimens from 133 patients with laryngeal/hypopharyngeal squamous cell carcinomas and 75 patients with laryngeal dysplasia, and correlated with clinical data. Our findings demonstrate that HERG1 is frequently aberrantly expressed in a high percentage of primary tumors (87%), whereas expression was negligible in both stromal cells and normal-adjacent epithelia. HERG1 expression increased during HNSCC progression and was significantly associated with lymph node metastasis ($p = 0.04$), advanced disease stages ($p < 0.001$), regional tumor recurrence ($p = 0.004$), distant metastasis ($p = 0.03$), and reduced disease-specific survival ($p = 0.012$, log-rank test). HERG1-positive expression was also detected in 31 (41%) of 75 laryngeal dysplasias. Interestingly, HERG1 expression increased with the grade of dysplasia; however, HERG1 expression but not histology correlated significantly with increased laryngeal cancer risk ($P = 0.007$). In addition, functional studies in HNSCC-derived cell lines further revealed that HERG1 expression promotes anchorage-dependent and independent cell growth and invasive capability, although independently of its ion-conducting function. Our data demonstrate that HERG1 expression is a biological and clinical relevant feature in HNSCC progression and also during malignant transformation and a promising candidate as cancer risk marker and therapeutic target for HNSCC prevention and treatment.

P13-31
Frequent aberrant expression of the human ether à go-go (hEAG1) potassium channel in head and neck cancer: pathobiological mechanisms and clinical implications

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Compelling evidence indicates that the human *ether*-à-*go-go* voltage-gated potassium channels (hEAG1) may represent new valuable membrane therapeutic targets and diagnostic/prognostic biomarkers in various cancers. This study is the first to investigate the expression of hEAG1 potassium channel subunit in both primary tumors and HNSCC-derived cell lines to ascertain its clinical and biological role in the progression of head and neck squamous cell carcinomas (HNSCC). Our findings demonstrate that hEAG1 is frequently aberrantly expressed in a high percentage of primary tumors (83%, 45/54 cases) and HNSCC-derived cell lines (83%, 10/12 cell lines). hEAG1 expression increased during HNSCC progression and was more frequent in advanced tumors. Strikingly, hEAG1 expression was also detected in a notable proportion (39%, 17/44 cases) of patient-matched normal adjacent mucosa, whereas no expression was detected in normal epithelia from non-oncologic patients without exposure to tobacco carcinogens. In an attempt to identify the underlying mechanisms of aberrant hEAG1 expression in HNSCC, we found that *hEAG1* gene copy gain occurred at a low frequency (15%, 13/88 cases) in primary tumors but was not observed in early stages of HNSCC tumorigenesis. Furthermore, this study provides original evidence supporting the involvement of histone acetylation (i.e. H3Ac and H4K16Ac activating marks) in the regulation of hEAG1 expression in HNSCC. In addition, functional studies in HNSCC cells further revealed that hEAG1 expression is a biologically relevant feature that promotes cell proliferation and invasion, although independently of its ion-conducting function. Our findings strongly support the notion that hEAG1 may represent a promising candidate as tumor marker and membrane therapeutic target for HNSCC treatment.

P13-32
Cortactin and focal adhesion kinase: novel markers of laryngeal cancer risk in patients with laryngeal premalignancy

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CTTN and *FAK*/*PTK2* gene amplifications were respectively detected in 10 (12%) and 26 (32%) laryngeal dysplasias. Both CTTN and FAK protein expression increased with the grade of dysplasia; however, CTTN and FAK expression but not histology correlated significantly with increased laryngeal cancer risk ($p = 0.009$ and $p = 0.002$, respectively). Patients carrying strong CTTN- or FAK-expressing dysplastic lesions experienced a significantly higher cancer incidence ($p = 0.006$ and $p = 0.001$, respectively; log-rank test). Furthermore, FAK expression was an independent predictor of laryngeal cancer development (HR = 3.706, 95% CI = 1.735–7.916; $p = 0.001$) and the combination of FAK and CTTN showed superior predictive value (HR = 5.042, 95% CI = 2.255–11.274; $P < 0.001$). Taken together, our findings support the involvement of CTTN and FAK in malignant transformation and provide original evidence for their potential clinical utility as biomarkers for the risk of developing laryngeal cancer.

P13-33
Acute hepatopathy involves changes in the nucleocytoplasmic distribution of enzymes from the methionine cycle

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Methionine is metabolized by the methionine cycle, leading to the production of several key metabolites, among them S-adenosylmethionine (AdoMet) and homocysteine. Methionine adenosyltransferases (MATs), methyltransferases such as glycine N-methyltransferase (GNMT), S-adenosylhomocysteine hydrolase (SAHH), methionine synthase and betaine homocysteine methyltransferase (BHMT) are the main enzymes involved in this cycle. The enzymes of this route have been classically identified in the cytoplasm, and only recently some have been found in the nucleus (MATs, SAHH, GNMT). Here we present data that show changes in the nucleocytoplasmic distribution of these members of the cycle upon induction of acute hepatopathy. These studies also lead to the identification of BHMT in the cellular nucleus. In addition, the putative role of oxidative stress in the control of the nucleocytoplasmic distribution of both MAT I/III and BHMT has been explored using several agents (galactosamine, buthionine sulfoximine), and the putative protective effects of AdoMet, N-acetylcysteine and glutathione ethyl ester are been also analyzed. These studies may shed light on the pathophysiological implications of changes in the intracellular location of these enzymes.

P13r-34
Human Mesenchymal Stem Cells as a model for studying pathological mutations in mitochondrial DNA

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Human mitochondrial DNA (mtDNA) encodes for some essential proteins for the maintenance of the energetic homeostasis, like some subunits of the oxidative phosphorylation system (OXPHOS). It also encodes for different RNAs (rRNAs and tRNAs) required for the expression of these proteins. Mutations of mtDNA are associated with a wide spectrum of disorders. In order to explore the impact of mitochondrial mutations on cell biochemistry and physiology, since 1991, a very interesting model has been used to rule out possible environmental or genetic (produced by the nuclear genome) influences. This model is known as transmitochondrial cell lines, cytoplasmic hybrids or just cybrids and constitutes a widely used tool in mitochondrial genetics studies. Cybrids are cell lines originated from the fusion of nucleated cells without mtDNA (rho0), and enucleated cells like cytoplasts or

platelets. However and due to the tumoral origin of the rho0 cell lines, some considerations should be taken when using this model, like the altered differentiation capacity and karyotype. To deal with this, we must search for another potentially useful cell source for building cybrids, like Stem cells, which are generally a source of euploid cultured cells. In this work, we characterize the mitochondrial function of a human mesenchymal stem cell line (hMSC) in two states, undifferentiated and differentiated into adipocytes, as a starting point to further analyze the influence of the mitochondrial genome in the auto-renewal cell capacity and differentiation potential, necessary to maintain the tissue dynamic. This phenomenon and how it could contribute to the appearance of a pathologic phenotype must be well understood before this kind of cells could be used to study the effects of mutations on mtDNA.

P13r-35
Dysregulation of GSK3β-dependent JunB degradation causes chromatid cohesion defects in ALK-positive anaplastic large cell lymphoma

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JunB, a member of the AP-1 (activator protein 1) family of dimeric transcription factors, plays an important role in cell proliferation acting either as a tumor suppressor or an oncogene depending on the cell context. JunB is strongly up-regulated in Anaplastic lymphoma kinase (ALK)-positive Anaplastic large cell lymphoma (ALCL), an aggressive type of non-Hodgkin lymphoma. Although its overexpression is linked to lymphomagenesis, the mechanisms whereby JunB promotes neoplastic growth are not fully understood.

JunB is degraded before mitosis by the ubiquitin-proteasome system. We characterized a critical consensus phospho-degron that controls JunB turnover and identified GSK3 and SCF^{FBXW7} as, respectively, the kinase and the E3 ubiquitin ligase responsible for its degradation in G2. Impaired JunB degradation in late G2 resulted in transcriptional repression of DDX11, a DNA helicase involved in the regulation of the G2/M transition. Cells with repressed levels of DDX11 displayed sister chromatid defects, underlining that physiological degradation of JunB in late G2 is critical for proper mitosis.

In ALK-positive ALCLs, inactivation of GSK3β mediated by the ALK/PI3K/AKT signaling pathway contributes to JunB protein stabilization and DDX11 repression in G2/M, resulting in chromatid cohesion defects. As chromatid cohesion is an essential step in maintaining genetic fidelity during mitosis, our results describe a new mechanism whereby JunB may contribute to tumorigenesis.

P13-36
Role of G protein coupled receptors in the adhesion phenotype of anaplastic large cell lymphoma cells

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CD30 is a receptor whose overexpression is characteristic of Hodgkin's Disease as well as systemic Anaplastic Large Cell Lymphoma (ALCL), an aggressive cancer arising from T cells and accounting for ~15% of all non-Hodgkin lymphomas in children and 5% in adults. CD30 is a member of the tumor necrosis factor (TNF) receptor superfamily. Engagement by its ligand CD30L results in the recruitment of TNF-R associated factors (TRAFs). CD30 activation can result in either proliferative or apoptotic downstream events. Signals emanating from CD30 can diverge at TRAF-2 & 5, leading to either proliferation via NF-κB and c-Jun or cleavage of caspases and subsequent apoptosis. We have observed that treatment of ALCL cell-lines with recombinant human CD30L results in 90% of the cells becoming adherent following growth in conditioned medium. In addition, we found that 7–13% of cells treated in this way die and have demonstrated using caspase 3 staining and flow cytometry that this results from apoptosis. We have compared the transcriptional profiles of ALCL cells cultured for 24 hours in either fresh-medium or conditioned medium, with and without the addition of CD30L, using Affimetrix U133A arrays. Exclusion of all transcripts with less than a 2-fold change in expression resulted in 3550 genes of interest. Partitioning around medioids clustering revealed 387 genes of interest that were differentially regulated in cells cultured in conditioned medium with the addition of rhCD30L. The twelve most up-regulated genes in this group include a number involved in GPCR signaling. To validate the observed micro-array data, quantitative real-time PCR was performed. We are currently testing the effects of siRNA-mediated knock-down of members of the GPCR family to assess their involvement in the expression of the adhesive phenotype. A detailed understanding of CD30 signalling in ALCL cell-lines and knowledge of how these cells respond to the extracellular environment increases our potential to develop novel and appropriate therapies for this devastating condition.

P13-37
Mutations in KCND3 gene cause spinocerebellar ataxia type 19

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Autosomal dominant spinocerebellar ataxia type 19 (SCA19) is characterized by a late-onset, slowly progressive, mild cerebellar

ataxia, hyporeflexia, postural head tremor, myoclonic movements, and progressive cognitive impairments. In 2002, we mapped the SCA19 disease gene to chromosome region 1p21-q21 using linkage analysis. We performed exome sequencing in two affected members of the Dutch SCA19 family and found one novel missense mutation that led to a c.1054A > C; p.T352P change in the *KCND3* gene. In addition, we screened the coding region of *KCND3* in 230 Dutch autosomal dominant ataxia cases and we identified two new missense mutations in *KCND3*, c.1119G > A; p. M373I and c.1169G > A; p.S390N, in two different ataxia families. All of these mutations change highly conserved amino acids in Kv4.3 protein, a voltage-gated potassium channel involved in the transient A-type K^+ currents in cerebellar neurons. Immunohistochemisty in SCA19 autopsy cerebellum, carrying the T352P mutation, showed a significant loss of Purkinje cells and altered localization of mutant Kv4.3 protein, as well as decreased protein expression levels. All mutated proteins showed endoplasmatic reticulum retention in transfected HeLa cells and reduced protein stability. Interestingly, proper plasma membrane localization of Kv4.3 could be rescued by co-expressing T352P and M373I mutants with their regulatory β-subunit KChIP2, but not the S390N mutant. Single cell patch-clamp experiments were performed to test the functionality of the mutated channels. We observed a strong reduced activity for T352P-KChIP2, S390N-KChIP2 and M373I-KChIP2 channel complexes (1%, 13% and 25% respectively compared to wild-type Kv4.3-KChIP2 (100%)). Whether the mutant kv4.3 subunits affect wild-type Kv4.3 is currently being investigated. Defects in ion channels seem a common mechanism underlying many of the SCA types and highlight the importance of identifying new disease genes as they can advance our understanding of the disease etiology.

P13-38
Association of CYP1A1 and CYP1B1 genes polymorphisms with breast cancer in Kazakhstan population

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Association of CYP1A1 and CYP1B1 Genes Polymorphisms with Breast Cancer in Kazakhstan Population The cytochromes P450 (CYPs) play an important role in cancer development. Breast cancer is the most common malignancy in women and its rate is growing in Kazakhstan Republic nowadays. Associations of single-nucleotide polymorphisms in *CYP1A1* (rs4646903) and *CYP1B1* (rs1056836, CYP1B1*3, or Leu432Val) genes with the development of breast cancer were studied in two main ethnic groups of Kazakhstan: Kazakhs-Mongoloids (121 patients, 218 controls) and Russians-Caucasians (60 patients, 179 controls). All samples were genotyped by polymerase chain reaction (PCR) based on restriction fragment length polymorphism (RFLP). Statistical significance of intergroup differences was assessed by means of the Pearson $\chi 2$ or Fisher exact test as dictated by statistical reasons. Testing of the *CYP1A1* gene rs4646903 polymorphism in Kazakh group showed the statistically significant differences in allele frequencies (OR = 1.51; 95% CI = 1.08–2.10; p = 0.004) and genotypes distribution (OR = 2.69; 95% CI = 1.35–5.36; p = 0.015) between patients and controls. No differences were observed in Russian ethnic group in *CYP1A1* gene. The genotypes distribution in *CYP1B1* gene revealed the statistically significant difference in Russians (heterozygote comparison: OR = 2.08; 95% CI = 1.15–3.77, p = 0.023; dominant model comparison: OR = 2.87; 95% CI = 1.07–7.67, p = 0.03). No significant differences were found in rs4646903 of *CYP1B1* gene in Kazakhs. All investigated groups were in HWE. The data presented suggest that G allele of the *CYP1A1* gene polymorphic locus rs4646903 can be considered as a risk factor for the development of breast cancer in Kazakhs but not in Russians in Kazakhstan.

P13m-39
Increased VLDL-triglyceride secretion rate is not sufficient to compensate hepatosteatosis in Glycine N-methyltransferase deficient mice

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Background: Chronic changes in S-adenosylmethionine (SAMe) availability in liver induce spontaneous development of non-alcoholic fatty liver disease (NAFLD). In MAT1A-KO mice, hepatic SAMe deficiency decreased VLDL-triglyceride (TG) secretion, which could contribute to the hepatosteatosis. Mice lacking Glycine N-methyltransferase (GNMT) show increased availability of SAMe in liver and develop different stages of NAFLD. In this context our aim was to investigate if chronic excess of hepatic SAMe modulates VLDL secretion in GNMT-knock out (KO) mice.

Methods: Three-month-old GNMT-KO mice and their wild type (WT) littermates were fed a control diet (CD) and a methionine-deficient diet (MDD) for 3 weeks. Newly secreted VLDL particles were isolated by ultracentrifugation from serum after 6 hours inhibition of their metabolism by poloxamer-407. VLDL size, apolipoprotein and lipid content were analyzed. Liver microsomes were isolated by ultracentrifugation. Lipid content in microsomes was quantified, and VLDL assembly related enzymatic activities were measured by radiometric assays.

Results: VLDL-TG secretion was increased while -apoB100 secretion was diminished in GNMT-KO mice as compared to their WT. This made VLDL size enlarged in GNMT-KO mice raising from 82,9nm in WT to 101,6nm in KO. In addition, impaired VLDL secretion in GNMT deficient mice was linked to altered microsomal lipid content, where TG, Diglyceride (DG) and phosphatidylcholine were increased and phosphatidylethanolamine was decreased. Regarding activities involve in VLDL assembly, DG acyl transferase activity was increased in KO mice in comparison with WT mice while TG lipase activity remained unaffected. Control VLDL features and microsomes lipid composition were restored when KO animals were fed a MDD.

Conclusion: The increased rate in VLDL-TG secretion, mainly due to higher chronic SAMe availability in liver, is not sufficient to alleviate hepatic steatosis in GNMT-KO mice.

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P13r-40
Rab14/Akt pathway is usurped by *Chlamydia trachomatis* to ensure its development

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Chlamydia trachomatis, an obligate intracellular pathogen, causes the most frequent sexually-transmitted bacterial disease worldwide. Chronic infections can result in female infertility. These bacteria exploit different trafficking pathways to acquire nutrients essential for their survival. We have demonstrated that Rab14, a key regulator of vesicular transport between the Golgi apparatus and early endosomes, is recruited to the inclusion and is involved on sphingolipids delivery from the Golgi to the inclusion. On the other hand, it has been described that Akt, a Ser/Thr kinase, phosphorylates AS160, a GAP (GTPase Activating Protein) for Rab14. The phosphorylation of AS160 results in the inhibition of its GAP activity, leaving Rab14 in its active state bound to GTP. We postulate that *C. trachomatis* might manipulate PI3K/Akt pathway to ensure the arrival of sphingolipids delivered from the Golgi to the inclusion through Rab14-positive vesicles. We analyzed the effect of a specific AKT inhibitor (iAkt) on HeLa cells infected with *C. trachomatis*. Treatment with iAkt decreased chlamydial inclusion size, inhibited homotypic fusion and reduced Rab14 recruitment to the inclusion in a doses-dependent manner. Moreover, iAKT treatment of infected cells retains sphingolipids inside Golgi apparatus assessed by confocal microscopy and generated abnormal bacterial forms observed by electron microscopy. Coincidently, these aberrant bacteria were likely observed within sphingomyelin-deficient cells and in persistent infections. Likewise, inclusion forming unit analysis clearly showed that iAKT treatment significantly decreased bacterial multiplication and infectivity. These data suggest that *Chlamydia trachomatis* selectively usurps PI3K/Akt pathway to generate an intracellular niche favourable for its survival.

P13-41
NAD+ prevents I/R damage in fatty livers: modulation of the MPT by the SIRT3-CypD-ANT axis

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Given that fatty livers are considerably more susceptible to acute stressors, such as ischemia/reperfusion (I/R), and knowing that the incidence of this pathology is deeply increasing, there is an urgent need to find strategies against I/R injury (I/RI) in fatty livers. In this study, we wanted to establish if an acute treatment with NAD+, a sirtuin cofactor, prevents mitochondrial dysfunction associated with warm I/RI in fatty livers. Zucker fatty rats were subjected to 120 min of 70% warm ischemia and 12 hours of reperfusion. In the treated group (NAD+) NAD+ was administered in the hepatic artery 30 min before ischemia. Acute treatment of livers before ischemia, decreased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) levels and also preserved mitochondrial cytochrome c content, comparatively to I/R livers. NAD+ also prevented calcium-induced mitochondrial permeability transition (MPT), the decline in mitochondrial respiratory state 3 and ATP content caused by I/R. The generation of reactive oxygen species (ROS) was also decreased in mitochondria isolated from I/R livers pre-treated with NAD+. Data shows that deacetylation of cyclophilin D (CypD) at Lys residue causes dissociation from the adenine nucleotide translocator (ANT), contributing to an increase in MPT threshold in NAD+-treated animals. This is due to a stimulation of the activity of mitochondrial SIRT3 that reduces the content in acetylated CypD in mitochondria from control and NAD+ livers, compared to the I/R group. Conclusion: These observations point CypD as a new protective target against I/RI in fatty livers. Pre-treatment with NAD+ protects the fatty liver by maintaining mitochondrial calcium homeostasis, thus preserving mitochondrial function and hepatic energetic balance.

P13-42
RasGRF1 regulates proliferation and metastatic behavior of human alveolar rhabdomyosarcomas

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The involvement of the Ras superfamily of GTPases in the pathogenesis of rhabdomyosarcoma (RMS) is not well understood. While overexpression on H-Ras leads to embryonal RMS (ERMS) formation in experimental animals and in Costello syndrome patients, no data exists on potential role of Ras GTPases in pathogenesis of alveolar RMS (ARMS). To address this issue better, we focused on the role of the GTP exchange factor RasGRF1 in this process. We observed that, in comparison to normal skeletal muscle cells, RasGRF1 mRNA is upregulated in all 8 human ARMS cell lines and subsequently confirmed its high expression in patient samples. By employing confocal microscopy analysis, we observed RasGRF1 accumulation in cell filopodia, which suggests its involvement in ARMS cell migration. Furthermore, we observed that RasGRF1 becomes phosphorylated in ARMS after stimulation by several pro-metastatic factors, such as SDF-1 and HGF/SF, as well as after exposure to growth-promoting IGF-2 and Insulin. More importantly, activation of RasGRF1 expression correlated with activation of p42/44 MAPK and AKT. When the expression of RasGRF1 was downregulated in ARMS cells by employing an shRNA strategy, these RasGRF1-kd RMS cells did not respond to stimulation by SDF-1, HGF/SF, IGF-2, or insulin by phosphorylation of p42/44 MAPK and AKT and lost their chemotactic responsiveness; however, they retained their pro-adhesive properties. We also observed that RasGRF1-kd ARMS cells proliferated at a very low rate *in vitro*, and, more importantly, after inoculation into immunodeficient SCID/beige inbred mice they formed significantly smaller tumors. We conclude that RasGRF1 plays a pivotal role in ARMS pathogenesis and is emerging as a new potential therapeutic target to inhibit ARMS growth.

P13-43
Deletion of Fn14 receptor protects from right heart fibrosis and dysfunction

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Rationale: Pulmonary arterial hypertension (PAH) is a fatal disease for which no cure is yet available. The leading cause of death in PAH is right ventricular (RV) failure, characterized by RV hypertrophy and fibrosis. Previously, the TNF receptor superfamily member Fn14 has been associated with different fibrotic diseases and left ventricular (LV) hypertrophy. However, there is so far no study demonstrating a causal role for endogenous Fn14 signaling in LV or RV heart disease.
Objective: The purpose of this study was to determine whether global ablation of Fn14 prevents RV remodeling improving right heart function.
Methods and results: Here, we provide evidence for a causative role of Fn14 in pulmonary artery banding (PAB)-induced RV fibrosis and dysfunction in mice. Fn14 expression was increased in models of RV pressure overload (PAB, monocrotaline) in the right, but not left ventricle. Mice lacking Fn14 ($Fn14^{-/-}$) displayed substantial reduction in RV fibrosis and dysfunction following PAB compared to wild-type littermates. Cell culture experiments demonstrated that activation of Fn14 induces myofibroblast differentiation and collagen expression via RhoA-dependent nuclear translocation of MAL/MRTF-A. Furthermore, our findings suggest ET-1 as a mediator of TWEAK/Fn14 profibrotic actions in cardiac fibroblasts. Activation of Fn14 *in vitro* caused complimentary to fibroblast proliferation a marked increase in cardiomyocyte size, which corresponds to suppression of PAB-induced RV hypertrophy in $Fn14^{-/-}$ mice.
Conclusion: The Fn14-RhoA-MAL axis is a key regulator in RV fibrosis and identifies Fn14 as potential new RV-specific target for therapeutic interventions.

P13-44
Effects of Salmonella enteritidis serovar typhimurium infection in adenocarcinomic human alveolar basal epithelial cells A549: pathogen induces apoptosis

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Salmonella enterica serovar Typhimurium is a facultative anaerobic bacteria, gram-negative, flagellated which generally develops and grows preferably in a wide variety of tumor cells. Until now several factors for this predilection have been described, such as: a increase number of nutrients favorable for bacterial growth due to rapid growth of the tumor cells, adaptation of *S. typhimurium*, loss of macrophages and neutrophils bactericidal activity in areas of hypoxia, the absence of antibodies and complement factors around the tumor. A549 cells are human alveolar basal epithelial cells. These are squamous in nature and responsible for the diffusion of substances, such as water and electrolytes, across the alveoli of lungs. They grow adherently, as a monolayer, *in vivo*. This study analyzed the infection of *Salmonella enteretidis serovar typhimurium* in adenocarcinomic human cells in three different aspects: morphological characterization of the infection, analysis of cellular DNA degradation and production of cytokines such as TNFα, IL-06 and IL-08. The morphological assay showed tumor cell shrinkage, nuclear fragmentation and chromatin condensation suggesting that the bacteria was inducing apoptosis on the adenocarcinogenic epithelial cells. For further investigation we analyzed if there was occurring the DNA degradation of the tumor cells, and it was seen. The quantification of cytokines was performed by comparison of mRNA production between the inner control (GAPDH) present in every homeostatic cell and the target molecules. Such methodology showed great variations among the strains analyzed and some predictable results, for example, bacterial strains isolated from diarrhea were more virulent against the tumor cells than others. In conclusion, we have shown some data proving that *Salmonella enteritidis serovar typhimurium* is able to adhere and infect adenocarcinomic human alveolar basal epithelial cells causing the human cell to undergo programmed cellular death. A pattern was established, the more virulent the strain the higher the quantity of cytokines produced in the inflammatory process. Therefore this study opens a new field for this kind of tumor treatment using, for example, the mutated for some virulence factors bacteria as a more precise delivery system of any chemotherapeutic agent.

P13-45
Polymorphism of Glutation-S-Transferase (GSTM1, GSTT1, GSTP1) genes in breast cancer patients of Kazakhstan

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Glutation-S-transferases (GSTs) are enzyme of phase II xenobiotics metabolism. It has been postulated that these enzymes play an important role in carcinogens disruption and elimination. We had performed the case-control study of *GSTM1*-null, *GSTT1*-null, and *GSTP1* (105 Ile/Val-A313G) polymorphisms in two main ethnic groups – Kazakhs (120 patients, 220 controls) and Russians (60 patients, 177 controls) of Kazakhstan Republic (Central Asia). Genotyping analyses of *GSTM1*, *GSTT1* and *GSTP1* were conducted by means of PCR (polymerase chain reaction). Statistical significance of intergroup differences was assessed by means of the Pearson χ^2 or Fisher exact test as dictated by statistical reasons. All investigated groups were in HWE. No significant differences were registered in both ethnic groups for *GSTM1* and *GSTT1* copy number variations polymorphisms. Any association with breast cancer risk was not revealed by two-way combination of *GSTM1*-null and *GSTT1*-null polymorphisms. Significantly increased risk for breast cancer was found in group of Kazakh patients with G allele (OR = 1.50; 95% CI = 1.05–2.16; p = 0.007) and GG genotype of *GSTP1* gene (OR = 2.38; 95% CI = 0.96–5.91; p = 0.076). *GSTP1* polymorphism was not associated with breast cancer risk (for GG genotype OR = 2.07; 95% CI = 0.71–6.09; p = 0.351) in Russian group. Thus, *GSTP1* (105 Ile/Val-A313G) polymorphism can be regarded as potential risk marker of breast cancer in Kazakh ethnic group.

P13-46
Analysis of muscle damage induced by a concentric exercise until volitional failure

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Introduction: Exercise-induced muscle damage is widely described in literature. Using different blood markers of muscle damage it would be feasible to determine the overall magnitude of injury into the muscle, as well as the type of fibres affected.
Materials and Methods: Physical education students performed an exercise test in a pneumatic leg press Air300 (Keiser, AR, USA). The test consisted in 10 sets with repetitions until exhaustion at workload equivalent to 75% of 1RM. The power of each repetition was recorded using a linear encoder integrated to Musclelab 4020e (Ergotest, Norway) system. Three blood samples were taken (1: before exercise test; 2: 24 hours post exercise; 3: 48 hours post exercise) of each subject to analyze the plasmatic concentration of myosin muscle isoforms and CK. ELISA method was used to determine myosin concentration in plasma. This technique has recently been adapted by our lab to assess fast and slow myosins.
Results: A significant increase was found 24 hours post-exercise in plasma levels of CK. In contrast, a different pattern was shown in myosin isoforms. While there was a significant increase of fast myosin in those subjects that showed a decrease of power during the test, slow myosin was raised significantly in participants that were able to maintain power constantly during the overall effort.
Conclusions: High plasma levels of CK indicated a muscle damage which would be associated with an increase of membrane permeability. The plasmatic changes found in both isoforms of myosin, fast and slow, were related with the exercise pattern performed by subjects. Interestingly, this fact may explain a major or less recruitment of the different types of muscle fibres during the physical exercise.

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Keywords: muscle damage, myosin slow and fast.

P13-47
Mechanisms of multipotent mesenchymal stromal cells effects on inflammation under pyelonephritis

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Pyelonephritis is an infectious-inflammatory disease caused by ascending infection. This pathology may lead to the chronic kidney failure. Pyelonephritis therapy is ineffective now, and investigation of alternative treatment methods, including stem cells therapy, are carrying out intensively. Multipotent mesenchymal stromal cell (MMSC) are believed to have immunomodulatory effects and to enhance repair by secreting anti-fibrotic and pro-angiogenic factors. Thus, MMSC could be applicable to treat acute pyelonephritis. In this study acute pyelonephritis in rats was made by introduction of bacterial suspension into urinary bladder. Development of inflammation was observed: concentration of leukocytes in urine and blood increased, especially neutrophils. We revealed activation of reactive oxygen species (ROS) production in leukocytes. Subsequent development of oxidative stress in the kidney was observed by increase of malonic dialdehyde level. Moreover, increase of nitric oxide (NO) and tumor necrosis factor (TNF) synthesis were revealed. We found that histopathological signs of inflammation and damage of kidney tissue were decreased after MMSC treatment. We conclude that MMSC have immunomodulatory properties and may diminish inflammatory process and kidney tissue injury. For analysis of MMSC functioning in proinflammatory environment, MMSC were cocultivated with leukocytes activated by lipopolysaccharide. We observed increase of ROS and NO production, increased levels of inducible NO-synthase, matrix metalloprotease-2 and phosphorylated glycogen synthase kinase-3 in MMSC after cocultivation. We concluded that leukocytes preconditioned MMSC by secretion of pro-inflammatory cytokines. Remarkably, preconditioned MMSC decreased inflammation in higher degree, than control MMSC. These results may open new therapeutic possibilities of MMSC in acute pyelonephritis and other acute and chronic inflammatory renal diseases.

P13-48
LMTK2 facilitates CFTR endocytosis by phosphorylation at CFTR Ser-737 residue

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Cystic Fibrosis, the most common lethal autosomal recessive disease in Caucasians, is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. CFTR functions as a cAMP-activated Cl- channel at the apical membrane of epithelia, where its membrane abundance is controlled by clathrin-mediated endocytosis, a mechanism that is only partially understood. Lemur Tyrosine Kinase 2 (LMTK2), a transmembrane protein with serine/threonine kinase activity, phosphorylates *in vitro* Ser-737 in the regulatory region of CFTR. LMTK2 controls intracellular trafficking and binds directly to myosin VI, a motor protein that facilitates CFTR endocytosis. Our aim was to determine whether LMTK2 facilitates CFTR endocytosis in human airway epithelial cells (CFBE41o- and Calu-3). Our data showed that endogenous LMTK2 co-immunoprecipitated with CFTR. Silencing LMTK2 increased the plasma membrane abundance of CFTR by attenuating CFTR endocytosis. An LMTK2 fragment containing the transmembrane and kinase domain deficient in kinase activity (TM + KD-K168M) inhibited the plasma membrane abundance of CFTR and attenuated CFTR endocytosis compared to control. Taken together, our results demonstrate that LMTK2 facilitates CFTR endocytosis in human airway epithelial cells by a mechanism requiring phosphorylation of Ser-737 in CFTR. Previously published data demonstrate that phosphorylation of Ser-737 inhibits CFTR mediated Cl- currents. Our results reveal that such Ser-737–dependent inhibition of CFTR-mediated Cl- currents is due, at least partially, to LMTK2-dependent CFTR endocytosis. Work supported by NIH-R01HL090767, R01HL090767-02S1 (ASU), FCT-PIC/BIA-BCM/112635/2009, BioFig PEst-OE/BIA/UI4046/2011 and SFRH/BD/47445/2008 fellowship to SL. SL is recipient of a FEBS Bursary.

P13-49
The effect of kiwifruit cysteine protease actinidin on the epithelial integrity and the expression of proinflammatory cytokines in T84 human colonic cells

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Background: Kiwifruit is among the first 10 food allergen sources in some European countries. Actinidin is a cysteine protease and a marker allergen for genuine sensitization to kiwifruit, probably because of its high abundance in kiwifruit and its potential to reach the intestinum partially undegraded. The aim of this study was to investigate if actinidin affects the epithelial integrity and the expression of proinflammatory cytokines in T84 human colonic epithelial cells
Method: Actinidin was isolated and purified from fresh kiwifruit using standard chromatographic methods. The transepithelial resistance (TER), the passage of Blue Dextran (BD) dye and the leakage of actinidin and thaumatin-like protein across the T84 monolayer were measured as epithelial integrity parameters. The mRNA expression of IL-6, IL-8, IL-1β and TNFα was performed by reverse-transcriptase polymerase chain reaction. Analysis of variance was used to assess the statistical significance of the obtained data.
Results: After 4 hours of actinidin treatment, the TER was reduced to 77 ± 3% ($p < 0.001$), and the BD level in the basolateral compartment increased to 20 ± 3% ($p < 0.01$). Translocation of actinidin and thaumatin-like protein across the T84 monolayer was detected by immunoblot. Quantitative assessment of the leakage of actinidin revealed that around 14.5 ± 0.6% of apically applied actinidin was found in the basolateral compartment. Actinidin also upregulated the expression of IL-1β mRNA in T84 cells ($p < 0.001$), which is known to contribute to epithelial integrity breach and inflammation. None of the observed effects were detected after treatment with inactivated actinidin indicating that the processes were protease-dependent.
Conclusion: The data observed in this study contribute to the understanding of intestinal sensitization mechanisms to kiwifruit and should help in designing new therapeutic strategies for allergies to food sources which contain proteolitically active allergens.
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P13-50
Ethanol consumption alters protein degradation through TLR4 pathway in mice cerebral cortex

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Alcohol abuse induces brain damage and can lead to neurodegeneration. We recently demonstrated that ethanol is capable of promoting inflammatory processes in glial cells, upregulating cytokines and other inflammatory mediators through the activation of Toll-like receptor 4 (TLR4) signaling. Ethanol alters protein degradation processes in alcoholic liver pathology, but it is uncertain if the proteolytic processes participate in ethanol-induced brain damage. Herein, we evaluate whether the two major protein degradation mechanisms, ubiquitin proteasome system (UPS) and autophagy lysosomal pathway (ALP), are affected by ethanol treatment in the brain. To that end, wild-type (WT) and TLR4 deficient ($TLR4^{-/-}$) mice were treated chronically with 10% ethanol in water for 5 months. Protein extracts were obtained from mice cortices and the main proteins involved in both pathways were determined by western blot. Ethanol treatment increased the total amount of ubiquitinated protein as well as the expression of several subunits of the immunoproteasome (β2i, β5i, PA28α) in WT mice. However, the expression of the 20S constitutive subunits remained unchanged or was even downregulated (α2, β5). No significant changes were observed in ethanol-treated $TLR4^{-/-}$ mice. Conversely, ethanol induced downregulation of proteins related to the ALP (Atg12, Atg5, Cathepsin B, p62 and LC3) in WT mice, but not in $TLR4^{-/-}$mice. In summary, the present results provide the first evidence demonstrating that chronic ethanol treatment induces immunoproteasome activation and impairs ALP in cerebral cortex and that these events are dependent of TLR4 signaling. These findings could provide new insight into the mechanisms underlying ethanol-induced brain damage. (Supported by SAF2009-07503, PNSD, RED-RTA).

P13-51
Polyphosphate binds to human von Willebrand factor *in vivo* and modulates its interaction with Glycoprotein Ib

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Polyphosphate, a phosphate polymer released by activated platelets, has been recently described as a potent modulator of blood coagulation and fibrinolysis. In blood plasma, polyphosphate binds and alters the biological functions of factor XII, fibrin(o-gen), thrombin, and factor VII activating protease.The aim of the present study is to investigate whether polyphosphate also binds to von Willebrand factor (vWF) and alters some of its activities. Studying patients with type 1 von Willebrand disease (vWD) and their healthy relatives, we discovered a significant correlation between von Willebrand factor (vWF) and platelet polyphosphate levels. We have also found polyphosphate in preparations of vWF isolated from normal platelets and plasma. Surface plasmon resonance and electrophoretic mobility assays indicated that polyphosphate interacts with vWF in a dose- and time-dependent manner. Treatment of normal plasma with active exopolyphosphatase decreased the vWF ristocetin cofactor (vWF:RCo) activity, a functional measure of vWF binding to platelet glycoprotein receptor Ib. vWF collagen binding and multimerization were unaltered after polyphosphate depletion. Moreover, addition of polyphosphate increased the defective VWF:RCo activity present in plasma from patients with type 1 VWD. Our results reveal that a new role is played by polyphosphate in hemostasis by its interaction with VWF, and suggest that this polymer may be effective in the treatment of some types of VWD. This work was supported in part by the EU (FEDER) and the Spanish Ministry of Science and Innovation (Grant FIS/PI10/01222 to F.A.R.), the Junta de Andalucia (Grants P07-CTS-02765 and C.S.0257/09 to F.A.R.), and Plan Andaluz de Investigacion (Cod. CTS-554).

P13-52
β-lactam antibiotics increase complement mediated immunity and enhances bacterial clearance of antibiotic-resistant pneumococcal strains

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Background: Prophylactic measures against *S. pneumoniae* are an effective strategy to prevent Invasive Pneumococcal Disease (IPD). The successful outcome against IPD, largely depends on the host immune system and the effectiveness of the antimicrobial chemotherapy. The aims of this study were to explore the ability of cefditoren to increase C3b, C1q and CRP deposition on the surface of different pneumococcal resistant strains opsonized with serum containing pneumococcal antibodies and investigate whether cefditoren might accelerate bacterial clearance from blood in the presence of specific antibodies.
Methods: Three strains of serotypes 6B, 19F and 23F (MICs to cefditoren: 1, 2 and 4 μg/ml respectively) were used for this study. Sera containing pneumococcal antibodies were obtained from mice immunized against the different heat killed strains. C3b, C1q and CRP deposition was analyzed by flow cytometry and bacterial clearance was investigated using a mice sepsis model.
Results: Deposition of C3b, C1q and CRP was significantly increased when the different isolates were incubated in the presence of 0.5MIC and 0.25MIC of cefditoren and serum containing specific antibodies. Administration of sub-therapeutic doses of cefditoren in immunized mice decreased significantly bacterial counts from blood during the first 24 h for all the strains investigated in comparison to the individual groups (lethal, antibiotic and passive immunization).
Conclusions: Antimicrobial chemotherapy with beta-lactams antibiotics such as cefditoren enhances bacterial killing in the presence of specific antibodies by activating complement mediated immunity against *S. pneumoniae*. Prophylactic strategies in combination with antibiotic treatment may overcome clinical failure by pneumococcal isolates with increased resistance.

P13-53
Sphingosine 1-phosphate induces inflammation and osteogenesis and increases the activity of the LPS/TLR4 route in human aortic valve interstitial cells

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Aims: Lipid accumulation in the aortic valve is a characteristic of aortic stenosis and sphingosine 1-phosphate (S1P) plays a relevant functional role in the cardiovascular system. On this basis, we investigated the possible role of S1P on the induction of pro-inflammatory and pro-osteogenic changes in human interstitial cells from aortic valve (AVIC) and pulmonary valves (PVIC).
Methods and Results: As regards pro-inflammatory routes, S1P up-regulated IL-6, IL-8, and cyclooxygenase (COX)-2 in AVICs, as determined by Western blot and ELISA experiments. AVIC exposure to a combination of S1P and bacterial lipopolysaccharide (LPS) a Toll-like receptor (TLR)-4 ligand known to promote pro-inflammatory and pro-osteogenic phenotypes in AVICs, resulted in the synergistic induction of COX-2, PGE_2, and intercellular adhesion molecule (ICAM)-1. Strikingly, the cooperative effect was stronger in stenotic than in control cells, and more prominent in cells from the aortic valves than from the pulmonary valves, which rarely undergo stenosis. Pharmacological and gene silencing experiments revealed the involvement of several S1P receptors in the synergistic effect. As regards pro-osteogenic processes, S1P induced the expression/activity of the calcification marker alkaline phosphatase (ALP) in *in vitro* calcification experiments. In addition, S1P cooperated with LPS to enhance significantly ALP activity and the cooperative effect was partially blocked by S1P receptor antagonists.
Conclusions: S1P induces pro-inflammatory and pro-osteogenic changes and increases the effect of TLR4 ligands in AVICs, what might be relevant for the pathogenesis of aortic stenosis and could open the way for new therapeutic approaches for this disease.

P13-54
SUMO-1 modified DJ-1 is translocated to the PML nuclear bodies

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Loss-of-function mutations in the *DJ-1* gene (PARK7 locus), are linked to PD. We searched for proteins interacting with DJ-1 by YTH screening in a human brain cDNA library. The screening resulted in the recovery of one novel DJ-1 interactor CHD3, and four previously identified interactors DJ-1, DJBP, PIASxα, and Ubc9. We showed that the capacity of DJ-1 to interact with these proteins was altered by the M26I and L166P pathogenic mutations. Both mutations impeded DJ-1 interaction with DJBP and additionally, the DJ-1^{M26I} mutant protein lost the interaction capacity with CHD3, and the DJ-1^{L166P} lost the capacity to form dimers with DJ-1^{WT}.

Using the UbFC fluorescence complementation assay we confirmed protein-protein interactions of DJ-1 with the E2 SUMO conjugation enzyme Ubc9, and with SUMO-1, and visualized the resulting protein conjugates in living cells by confocal microscopy. The DJ-1^{WT}/Ubc9 protein conjugates were homogenously distributed in the cytoplasm, and the DJ-1^{WT}/SUMO-1^{WT} conjugates were cytoplasmic with a speckled distribution in the nucleus. Interestingly, the sumoylation-deficient DJ-1^{K130R} mutant protein interacted with SUMO-1^{WT}, and the conjugation-deficient SUMO-1^{DelGG} mutant protein interacted with DJ-1^{WT}. These results indicate of a non-covalent interaction between DJ-1 and SUMO-1 and suggest that DJ-1 sumoylation results in DJ-1 translocation to Promyelocytic leukemia protein-Nuclear bodies (PML-NBs). Future studies should solve the functional consequence of DJ-1 localization at the PML-NBs, and investigate role of DJ-1 non-covalent interaction with SUMO-1 in the neurodegeneration process of PD. In addition we found a novel DJ-1 interactor, CHD3, which may underlie the mechanism by which DJ-1 regulates transcription of genes, namely those involved in apoptosis. Therefore a follow up study on this novel interaction may reveal important insights into the anti-apoptosis function of DJ-1.

P13-55
Reactive oxygen species levels in fibroblasts from patients with glycogen storage disease type IV

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Glycogen storage disease type IV (GSD IV) is caused by pathogenic mutations in glycogen branching enzyme gene (GBE1). Such enzymatic modification results in intracellular accumulation of poorly branched glycogen molecules characterized by low solubility and decreased glucose availability. It is not clear whether the observed cell injury results from mechanical damage of cellular structures due to presence of intracellular glycogen aggregates or if it is a consequence of energy metabolism dysfunction. Since dysfunctions in cellular energy metabolism are often associated with oxidative stress, we investigated the reactive oxygen species (ROS) levels in cells with different GBE1 mutations. We compared cytosolic and mitochondrial ROS levels in fibroblasts from control donors and from patients with GSD IV. Under basal conditions a tendency towards higher cytosolic ROS levels in fibroblasts from GSD IV patients was observed, however it did not reach statistical significance. Further studies will focus on changes in ROS levels in the investigated cells under different stress conditions.

P13-56
The analysis of the autoinducer synthesis genes involved in quorum sensing among biofilm forming *Bacillus cereus* isolated from food and clinical samples

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Bacillus cereus is a gram-positive, spore-forming food pathogen that cause diarrhea and emesis by ingestion of produced various toxins and is ubiquitously present in several environments. *B. cereus* can also form biofilm, multicellular complex, on various environmental surfaces such as food process equipments and clinical implants. Biofilm may serve as chronic source of microbial contamination. The biofilm-forming capacity of 88 emetic toxin producing *B. cereus* strains isolated from food and clinical samples was investigated in this study. A total of 34 (38.6%) of all strains formed biofilm. *B. cereus* strains (49%) from clinical samples more frequently formed than *B. cereus* strains (25.6%) from food samples. Whereas the strains from clinical samples showed lower biofilm-forming capacity than them from food samples. In the toxigenic profile study, all of biofilm-forming strains had non-hemolytic enterotoxin A (*nheA*), B (*nheB*), and C (*nheC*) genes but did not have hemolysin BL enterotoxin gene A (*hbl A*). Moreover, the biofilm-forming strains from clinical samples did not harbored one of *hbl* genes at all. To elucidate quorum sensing mechanism, we investigated mRNA expression and its quantification of autoinducer synthetic genes encoding signal substance of quorum sensing from biofim-forming *B. cereus, E. coli* and *S. aureus*. The three bacterial strains were grown in mono- and co-culture and the growth of three bacteria and the transcriptional level of *luxS* in each strains were monitored. The growth of *B. cereus* was significantly affected by co-cultivation. Transcriptional analysis showed that the expression of *luxS* from co-cultivation was higher than those from each mono-cultivation.

P13m-57
Resveratrol treatment restores peripheral insulin action in diabetic IRS2-deficient mice in a Sirt1-independent manner

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Mice with complete deletion of insulin receptor substrate (IRS) 2 develop hyperglycaemia, impaired hepatic insulin signalling and elevated gluconeogenesis. The elevated expression and activity of PTP1B in the liver of hyperglycaemic $IRS2^{-/-}$ mice block insulin receptor (IR)/IRS1-mediated insulin signalling. Accordingly, PTP1B inhibition by genetic (double mutant $IRS2^{-/-}PTP1B^{-/-}$) and pharmacological (resveratrol treatment) strategies promotes insulin sensitivity in the liver of $IRS2^{-/-}$ mice through the restoration of IRS1-mediated Akt/Foxo1 phosphorylation and the inhibition of gluconeogenic enzymes. Moreover, resveratrol, a plant-derived polyphenolic compound, is a potent activator of the histone deacetylase Sirt1. In fact, recent studies have demonstrated that both resveratrol treatment and moderate increase of Sirt1 levels improved insulin sensitivity. On that basis, the aim of this study was to investigate if resveratrol action on $IRS2^{-/-}$ mice is mediated by Sirt1 activation. For this goal, we have generated $IRS2^{-/-}$ mice with moderate over-expression of Sirt1. Resveratrol treatment improved systemic insulin sensitivity in hyperglycemic $IRS2^{-/-}$ mice but did not change glucose tolerance due to the inability to revert beta cell failure. Conversely, moderate over-expression of Sirt1 in $IRS2^{-/-}$ mice did recover neither peripheral insulin resistance nor glucose intolerance. In both liver and muscle of hyperglycaemic $IRS2^{-/-}$ mice levels of PTP1B were increased. Resveratrol treatment of $IRS2^{-/-}$ mice significantly decreased PTP1B mRNA and inhibited its activity in both tissues, thereby restoring IRS1-mediated insulin signalling. Conversely, moderate over-expression of Sirt1 could not normalize PTP1B levels and, consequently, insulin signalling remained impaired. Our results have established that elevated PTP1B expression in liver and muscle of hyperglycaemic $IRS2^{-/-}$ mice impaired insulin signalling. Moreover, we have demonstrated that the effects of resveratrol on insulin action in $IRS2^{-/-}$ mice are not mediated through Sirt1 activation.

P13-58
Analysis of promoter methylation of Casp8, Fas, BRCA1 genes in esophageal and cervical cancer

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Investigation of association between methylation and cancerogenesis is very important problem in modern molecular genetics. For a various of genes the methylation status in different types of tumor is determined. It is used as diagnostic and prognostic markers for cancer. We have investigated the methylation pattern of the promoters of genes involved in apoptosis regulation and control of genome integrity (Casp8, Fas, BRCA1) in normal and tumor tissues using methyl-sensitive PCR on cohorts of healthy

people and patients with cervical and esophageal cancer from Kazakh population.

Statistical analysis of the obtained data has shown that the methylation of the promoter of mutator gene BRCA1 (OR = 16.33; p = 0.006) and apoptosis gene Casp8 (OR = 21.46; p = 0.008) are significant risk factors of cervical cancer. Methylation of promoter of apoptosis inductor gene Fas isn't a risk factor for this oncopathology (OR = 1.20; p = 0.84).

Methylation of promoter of gene Fas (OR = 25.67; p = 0.003) and Casp8 (OR = 8.80; p = 0.02) are important in esophageal cancer development. Methylation of gene BRCA1 isn't associated with the risk of esophageal cancer development (OR = 1.18; p = 0.91).

Thus, different types of cancers are characterized by methylation of promoter in different genes. Methylation of the promoters of genes BRCA1 and Casp8 may be considered as potential biomarkers for cervical cancer and genes Fas and Casp8 – for esophageal cancer.

P13-59
The study of genetic factors in primary open-angle glaucoma patients in Latvia

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Glaucoma is the leading cause of irreversible blindness worldwide, affecting 2–10% of individuals over 50 years of age, and primary open-angle glaucoma (POAG) is the most prevalent age-related form of the disease. A major risk factor for POAG is an elevated intraocular pressure; however, the molecular events responsible for the disease onset and progression are currently poorly understood. Genetic factors are considered to be important contributors to the pathogenesis of POAG. To date, at least 14 genetic loci have been defined, and several genes that are associated or causal for glaucoma including *MYOC* (encoding myocilin) and *ANP* (encoding atrial natriuretic peptide) have been identified, although findings varied in different populations. The aim of this study was to determine the prevalence of mutations in the *MYOC* and *ANP* genes in Latvian patients with POAG. In addition, proANP level in aqueous humor and plasma of POAG and cataract patients was investigated. Results. Possible disease-causing mutations in *MYOC* gene, Gln368X, Arg422His, as well as a novel mutation Glu253Asp, were identified in 4% of Latvian POAG patients. Neutral polymorphisms of the *MYOC* gene were identified in 39% of studied POAG samples and 30% of cataract samples. None amino-acid-changing mutations were detected in the *ANP* gene in studied DNA samples. On the other hand, our results show significantly elevated proANP level in aqueous humor of POAG patients compared to cataract patients. Moreover, the proANP level was gradually increased in older POAG patients and patients with later POAG stages. Conclusions. Our findings demonstrate a possible association of *MYOC* gene and ANP with POAG in Latvian population. The research was supported by grants from the LCS No. 01.0010.01 and by ERDF project No. 2DP/2.1.1.2.0/10/APIA/VIAA/004.

P13-60
Interleukin 6 (IL-6) and neurotrophin 3 (NT-3) dynamics in patients with ischemic stroke (IS) and COPD

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The systemic inflammatory response induced by chronic and acute diseases are an important target for the actual research. The activation of microglial cells represents the key element of the inflammatory and neuroprotective response to acute hipoxia, furthermore the inflammatory cells present in COPD have been shown to play important role in production of pro-inflammatory cytokines and neurotrophins who have been recognized as mediators of both inflammatory and/or airway remodeling processes. The present work investigated the possible correlation of NT-3 with IL-6 and TNF-α from patients with IS and COPD. The patients were divided into two groups: group I including patients after IS with GOS 4 n = 20 (72.65 ± 13.72 years) and group II including patients having moderate COPD stage II GOLD, n = 20 (61.0 ± 14.19 years). NT-3, TNF-α and IL-6 serum level were determined using ELISA kits.TNF-α was detected in only 5 patients with IS GOS 4 with severe clinical course. An inverse correlation was found between NT-3 and IL-6 serum levels in group I (r = –0.61, p = 0.05). NT-3 was positive correlated with, TNFα (r = 0.93 p = 0.05) and IL-6 (r = 0.94, p = 0.05) in group II. The inverse correlation between NT-3 and IL-6 suggests that NT-3 may exert an immuno-modulatory action on IL-6 post TNF-α responses in patients survivor with acute IS. A positive correlation between these parameters in patients with COPD stage II GOLD corresponded to a chronic diseases. In the COPD pathogenesis depending on the context of local cytokines, NT-3 could be a mediator involved in airway inflammation.

P13-61
miRNAs as novel molecular markers for breast cancer diagnosis

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Breast carcinoma is the most frequent neoplasia among women in developed countries. Up to the moment, histopathological criteria have been used for diagnosis and classification, but these data do not reflect the biological variability of these tumors. Moreover, apparently homogeneous breast carcinomas demonstrate complete different behaviors and responses to therapies. These observations reveal that molecular differences among tumors could determine their evolution and responses to therapies. miRNAs are small non coding RNA molecules which regulate gene expression. They are involved in almost every cellular process and they have been pointed out as accurate biomarkers

for several diseases, including cancer. In this work, we have studied the expression of several miRNAs potentially involved in breast cancer development. Our aim is to identify a miRNA combination with potential molecular diagnostic and prognostic value for these tumors. For this purpose, we have determined by qRT-PCR the expression of miR-21, miR-221, miR-222, miR-342-3p and miR-520g in 50 biopsy samples of breast carcinoma patients (4 Her-2; 8 Basal-Like; 20 Luminal A; 15 Luminal B) and 5 healthy controls. Our results demonstrate significant changes between tumoral and non-tumoral breast tissue for miR-221, miR-222 and miR-520. Moreover, miR-342 exhibits significant differences in Luminal B tumors compared to other subgroups. These results strongly suggest that breast cancer subtypes can be classified attending to miRNAs expression. These molecular criteria might lead to more accurate diagnosis with potential important implication for breast cancer prognosis and treatment.

*Equal contribution to this work. $ Equal contribution as corresponding author to this work.

P13-62
mTORC1 hyperactivity: impaired mitophagy and macroautophagic response to endoplasmic reticulum stress

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Hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1) is usually found in progression to type 2 diabetes among other diseases. mTORC1 negatively regulates autophagy, a cytoprotective process, and its hyperactivity is also a well described cause of insulin resistance and endoplasmic reticulum (ER) stress. Our studies focused on the role that mTORC1 hyperactivation might be having on the selective autophagy of mitochondria (mitophagy), and the macroautophagic response under ER stress. For this purpose, we used mouse embryonic fibroblasts (MEF) $TSC2^{-/-}$, lacking the repressive action of TSC2 on mTORC1 signalling. $TSC2^{-/-}$ MEFs are widely used as model of chronic mTORC1 hyperactivation. Upon chemical ER stress stimulation, mTORC1 signaling is inhibited and autophagy stimulated, however $TSC2^{-/-}$ MEFs do not show mTORC1 downregulation, and levels of LC3B-II and autophagic puncta do not efficiently increase. Accumulation of p62/SQSTM1 is found basally on $TSC2^{-/-}$ MEFs, and is largely increased after ER stress stimulation. This behavior is completely reverted with rapamycin treatment, suggesting the key role of mTORC1 on autophagic stimulation after ER stress. As well, we focused on how the unfolded protein response signaling may be triggering autophagy. Likewise, chemical uncoupling of mitochondria triggers the specific elimination of uncoupled mitochondria by mitophagy. However, in $TSC2^{-/-}$ MEFs this response is impaired by mTORC1 hyperactivation, and only recovered after rapamycin treatment. We also sought to observe the effect that reduced macroautophagy and mitophagy might be having on cell survival. Here we provide the evidence of mTORC1 hyperactivation as a cause of heavily impaired autophagic response under ER stress, as well as abnormal autophagic clearance of mitochondria. Thus, showing the key role of proper mTORC1 signaling and the importance of autophagy for cell homeostasis.

P13-63
Mouse resistin modulates adipogenesis and glucose uptake in 3T3-L1 preadipocytes through the ROR1 receptor

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Mouse resistin, a cysteine-rich protein primarily secreted from mature adipocytes, is involved in insulin resistance and type 2 diabetes. Human resistin, however, is mainly secreted by immune mononuclear cells, and it competes with lipopolysaccharide for the binding to Toll-like receptor 4, which could mediate some of the well-known pro-inflammatory effects of resistin in humans. In addition, resistin has been involved in the regulation of cell differentiation and proliferation processes, suggesting that different receptors could be involved in mediating its numerous effects. Thus, a recent work identifies an isoform of Decorin (ΔDecorin) as a functional resistin receptor in adipocyte progenitors that may regulate white adipose tissue expansion. In this work, we have observed an interaction of mouse resistin with specific domains of the extracellular region of the mouse receptor tyrosine kinase-like orphan receptor 1 (ROR1). This interaction results in the inhibition of ROR1 phosphorylation, modulates ERK1/2 phosphorylation, and regulates the expression of suppressor of cytokine signaling 3, glucose transporter 4, and glucose transporter 1. We show also that the ROR1 receptor mediates the described effects of resistin in 3T3-L1 adipogenesis and glucose uptake. Our results identify mouse resistin as a potential inhibitory ligand for the ROR1 receptor and demonstrate, for the first time, that ROR1 plays an important role in adipogenesis and glucose homeostasis in 3T3-L1 cells. These data open a new line of research that could explain important questions about the mechanism of action of resistin in adipogenesis and in the development of insulin resistance and type 2 diabetes.

P13-64
Genes-partners on t-ANLL-associated chromosomal translocations exhibit different behavior in response to DNA topoisomerase II inhibition in lymphoid cells

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Type-1 diabetes (T1D) is an endocrine disorder caused by autoimmune destruction of pancreatic β cells, leading to uncontrolled chronic hyperglycemia. Complications related to T1D result in the dysregulation of several cellular pathways leading to target organ damages like diabetic cardiomyopathy, hepatic and renal failure and diabetic encephalopathy. A most used animal model of T1D is obtained by the administration of streptozotocin (STZ) to rats, thus giving the opportunity to tightly monitor the onset of the disease. The 14-3-3 family proteins are key signaling molecules, involved in the regulation of multiple cellular pathways, mainly the apoptotic one, consisting of seven distinct isoforms (β, γ, ε, ε, τ, η, σ), mainly localized in the cytosol. In this study we investigated the expression of 14-3-3 family proteins at transcript and protein levels in the brain and liver of short-term T1D rats. T1D was induced by STZ treatment and after 3-weeks puri-

fied brain and liver cytosolic proteins were prepared from control and T1D rats. The protein expression of all the seven 14-3-3 isoforms were studied by Western blot analysis using high-specific monoclonal antibody and the relative transcript levels were assessed by Real-time quantitative PCR. At brain level all the 14-3-3 isoforms showed a statistical significant changes in protein expression and mRNA level in T1D sample compared to control ones. In detail, six of the seven 14-3-3 isoforms (β, γ, ε, ε, τ, η) showed a marked decrease of both protein and mRNA content in the T1D brain, while for the 14-3-3 σ isoform the protein decreased level was opposite to the increased mRNA content in T1D rats. This finding could be attributed to post-translational events or to an enhanced binding of the protein with one of its multiple targets. On the other hand, in the liver of T1D rats only two 14-3-3 isoforms (β and γ) showed a statistical significant change, showing in this case an increment in both protein content and mRNA level. Overall our results indicate that the impact of short-term T1D on 14-3-3 proteins and transcript expression is different at brain and hepatic level. This might be associated with activation or inactivation of apoptotic pathways in a tissue-specific way.

P13-65
Relevance of melatonin in the immune response from peripheral lymphocytes of systemic lupus erythematosus patients

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Systemic lupus erythematosus (SLE) is a classic example of an aberrant immune response in which cytokine abnormalities have been implicated in the pathogenesis, both as part of pathogenetic core process of lupus or as secondary markers indicating immune dysregulation. Therefore, it is of great interest to find molecules that are able to modulate the inflammatory response restoring immune balance. In this line, the wide range of immunomodulatory properties described for melatonin (MLT) point it out as a potential T cells regulator through modulating of the cytokines production.

To evaluate the role of MLT in the modulation of T cells from SLE patients (n = 20) receiving antimalarial treatment, we studied the action of exogenous MLT in the production of cytokines hallmark of Th1/Th2 responses by ELISA and the endogenous MLT levels in stimulated peripheral blood mononuclear cells (PBMC) with PHA from SLE patients by bead based multiplex ELISA.

The entire patient population showed a significantly lower amount of cytokines (TNFα, IL6, IL2, IL10) in compared with healthy controls. Moreover, it was found that exogenous MLT modulates the cytokines production in SLE patients treated increasing cytokines production to levels similar to those in healthy controls. Finally, we observed that endogenous MLT levels from SLE lymphocytes were significantly lower than in healthy controls after stimulation, what may contribute to immune imbalance.

Although the mechanism underlying our findings is yet to be determined, our results suggest that the addition of melatonin would be able to play an important role in the immune responses of SLE patients.

P13-66
Putative Inhibitors of V-type ATPase decreases the virulence of *Candida albicans*

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Candida albicans is an opportunistic human pathogen. It has a unique ability that is changing its morphology, from yeast to hyphal growth in various stress conditions. This pathogen colonizes the skin and mucosal surfaces of infected individuals. The morphological conversion is believed to be one of the well known virulence factors in *C. albicans*. Through proteomic and genetic analysis, we showed that V-type ATPase is related with Candida virulence. Bafilomycin is a general inhibitor of ATPase having antifungal activity. Here, we designed the small molecules which are structural analogues of Bafilomycin which is known to be an inhibitor to target the V-type-ATPase. To characterize small molecules, we investigated the effect of small molecules on growth rate and the morphological transition. Growth and hyphal formation rate were decreased by treatment of small molecules. We found the appropriate concentration which has non-cytotoxicity in mammalian cells and an inhibitory effect on morphological transition in *C. albicans*. We also figured out the synergistic effect on morphological transition through combination treatments of various concentrations of these small molecules along with Fluconazole which has been used for the therapeutic treatment of candidiasis as an inhibitor of cytochrome P450 14α-demethylase. From these results, we suggest that several of small molecules have a great potential for antifungal activity.

P13-67
Haplotype analysis of the CAG and CCG repeats in 21 Brazilian families with Huntington's disease

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We studied the allelic profile of CAG and CCG repeats in 61 Brazilian individuals in 21 independent families affected by Huntington's disease (HD). After PCR amplification the allele profiles were determined on an ABI 3500 Genetic Analyser and the data were analysed using the GeneScan Analysis 3.7 and Genotyper 3.7 programs (Applied Biosystems). Thirteen individuals had two normal alleles for HD, two had one mutable normal allele and no HD phenotype and 46 patients carried at least one expanded CAG repeat allele. Forty-five of these individuals had one expanded allele and one individual had one mutable normal allele (27 CAG repeats) and one expanded allele (48 CAG repeats). Eleven of these 45 subjects had a mutant allele with reduced penetrance, and 34 patients had a mutant allele with complete penetrance. Inter- and intragenerational investigation of CAG repeats was also performed. We found a negative correlation between the number of CAG repeats and the age of disease onset ($r = -0.84$; $p < 0.001$) and no correlation between the number of CCG repeats and the age of disease onset ($r = 0.06$). This investigation of the CCG region and the CAG/CCG haplotypes is important

because it is the first study focused on Brazilian subjects. We found that CAG repeat expansion was predominantly associated with $(CCG)_7$, but, in two families, CAG repeat expansions were associated with $(CCG)_{10}$. Therefore, our findings indicate that HTT mutations likely have more than one ancestral origin.

P13-68
The proteins DLK1 and DLK2 modulate human SK-MEL-2 melanoma cell proliferation through notch signaling

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Notch receptors regulate cell proliferation and survival in several types of cancer. Notch1 can function as an oncogene or as a tumor suppressor gene depending on the cellular context. Recently, the EGF-like proteins DLK1 and DLK2 have been reported to interact with NOTCH1 and to inhibit NOTCH1 activation and signaling in different cell lines. It has also been reported that DLK1 is involved in the regulation of cell growth and cancer. However, nothing is known about the role of DLK2 in those processes. In this work, we focused on the role of DLK proteins and the NOTCH1 receptor in the control of melanoma cell growth, where NOTCH1 is known to exert an oncogenic effect. We found that over-expression of human DLK1 or DLK2 proteins promote growth of the human metastatic melanoma cell line SK-MEL-2, whereas decreased expression of DLK2 inhibits its proliferation. In addition, we observed that forced expression of DLK1 or DLK2 in these cells leads to a decrease in NOTCH1 activation and signaling, associated to diminished levels of HES-1, HEY-1 and HEY-2 expression. Both soluble and transmembrane DLK1 proteins were able to inhibit human NOTCH signaling in these cells. Furthermore, we show that NOTCH signaling in SK-MEL-2 cells is inhibited by DLK1 or DLK2 proteins in a dose-dependent manner. We observed that the growth rate of the cells depends on the level of NOTCH1 activation. In particular, high levels of NOTCH1 inhibition results in a decrease, whereas lower levels of NOTCH1 inhibition lead to an increase of cell proliferation. Taken together, our data suggest that DLK1 and DLK2 proteins are able to fine-tune the NOTCH1 signaling pathway and that this plays an important role in the control of melanoma tumor cell proliferation. NOTCH1 is an attractive target for cancer therapy. DLK proteins or derived peptides could be useful to help modulating NOTCH1 signaling in new target-based therapies for those tumors.

P13-69
Short-term Type-1 diabetes induces a tissue-specific expression of 14-3-3 family proteins

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Type-1 diabetes (T1D) is an endocrine disorder caused by autoimmune destruction of pancreatic β cells, leading to uncontrolled chronic hyperglycemia. Complications related to T1D result in the dysregulation of several cellular pathways leading to target organ damages like diabetic cardiomyopathy, hepatic and renal failure and diabetic encephalopathy. A most used animal model of T1D is obtained by the administration of streptozotocin (STZ) to rats, thus giving the opportunity to tightly monitor the onset of the disease. The 14-3-3 family proteins are key signaling molecules, involved in the regulation of multiple cellular pathways, mainly the apoptotic one, consisting of seven distinct isoforms (β, γ, ε, ε, τ, η, σ), mainly localized in the cytosol. In this study we investigated the expression of 14-3-3 family proteins at transcript and protein levels in the brain and liver of short-term T1D rats. T1D was induced by STZ treatment and after 3-weeks purified brain and liver cytosolic proteins were prepared from control and T1D rats. The protein expression of all the seven 14-3-3 isoforms were studied by Western blot analysis using high-specific monoclonal antibody and the relative transcript levels were assessed by Real-time quantitative PCR. At brain level all the 14-3-3 isoforms showed a statistical significant changes in protein expression and mRNA level in T1D sample compared to control ones. In detail, six of the seven 14-3-3 isoforms (β, γ, ε, ε, τ, η) showed a marked decrease of both protein and mRNA content in the T1D brain, while for the 14-3-3 σ isoform the protein decreased level was opposite to the increased mRNA content in T1D rats. This finding could be attributed to post-translational events or to an enhanced binding of the protein with one of its multiple targets. On the other hand, in the liver of T1D rats only two 14-3-3 isoforms (β and γ) showed a statistical significant change, showing in this case an increment in both protein content and mRNA level. Overall our results indicate that the impact of short-term T1D on 14-3-3 proteins and transcript expression is different at brain and hepatic level. This might be associated with activation or inactivation of apoptotic pathways in a tissue-specific way.

P13-70
Molecular mitochondrial (MT) pathology in eye diseases

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Symptoms of mitochondrial diseases (MD) may be acute or chronic with intermittent decompensation. Among the MD Leber's hereditary optical neuropathy (LHON) is characterized by the rapid, painless, bilateral loss of central vision and variable penetrance. Severity of disease depends on a particular MT DNA (mtDNA) defect, and underlying molecular mechanisms are likely to be more complex. The aim of the study was to investigate the role of MT mutations and their particular genotype in the phenotypic manifestation of visual loss. Seven patients were enrolled into the study, and five of them represented two families. Clinical examinations of patients were performed. The entire MT genome was amplified in 17 overlapping fragments and sequenced. mtDNA heteroplasmy was checked. MT haplogroups (HGS) were determined by restriction and by hypervariable segment I (HVSI) sequencing analyses. One common primary LHON-associated point mutation, G11778A (Arg → His) in the *MT-ND4* gene, was revealed in two members of one family. Visual acuity was ~0.3 and 0.4 for adult one and −0.1 for offspring. There was one secondary mutation found in the second family (mother, two daughters), A13637G (Gln → Arg) in the *MT-ND5* gene, but only mother exhibited clinical feature of LHON. The same mutation was detected in another male person, who suffered from moderate visual impairment. Different secondary mutation, T6253C (Met → Thr) in the *MT-COXI* gene, was found in 39-

year-old man. All the pathogenic mtDNA mutations were homoplasmic. mtDNAs of studied patients belong to HGS H and U5, which are the most abundant variants in Latvian population. Therefore, it is possible that MT HGS may influence the penetrance and expressivity of visual loss. Our study confirmed that alongside with primary LHON mutations, secondary mtDNA mutations may also be considered when giving genetic counseling. The research was supported by grants from the LCS no. 09.1284 and 01.0010.01.

P13-71
All dystroglycanopathies-causing genes are expressed in the retina of adult mammals

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Dystroglycanopathies (DGPs) are rare genetic neuromuscular dystrophies that offer a short life expectancy and whose severe symptoms extend to the retina. They include the Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB) and Fukuyama congenital muscular dystrophy (FCMD), among other milder disorders. Their causative genes encode enzymes involved in the O-glycosylation of α-dystroglycan (α-DG), a glycoprotein involved in basement membrane formation and the establishment of synapses in the nervous system. The existence of six genes has been unveiled so far, *POMT1*, *POMT2*, *POMGNT1*, *FKTN*/*fukutin*, *FKRP* and *LARGE*, all of which encode demonstrated or putative glycosyltransferases. However, the role of these genes and their protein products is not well established in the adult CNS, and their expression in the mammalian retina is mostly unknown.

We have evidenced by RT-PCR expression of *POMT1*, *POMT2*, *POMGNT1*, *FKTN*, *FKRP* and *LARGE* at the mRNA level in the adult neural retina of primates (human and monkey), cow and rodents (rat and mouse), as well as in the 661W photoreceptor cell line. Also, we have so far detected by Western blotting expression of POMT1, POMGnT1 and fukutin proteins in the monkey, bovine and rodent retinas, and in 661W cells. This analysis revealed bands for some of these proteins additional to that exhibiting the expected molecular weight, which could be encoded by non-canonical transcript variants. In this context, we have found that the *POMT1* mRNA undergoes alternative splicing in the human and bovine retinas. This has led us to subclone and sequence a series of alternative transcripts of the *POMT1* gene, which were found to exhibit skipped or inserted (extra) optional exons.

Our results are suggestive of a relevant role of DGP-associated genes not only in the brain and muscle, but also in α-DG glycosylation in the neural retina of adult mammals. Also, the expression of some of these genes in the retina could be regulated at the post-transcriptional level.

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P13-72
RNA interference (RNAi) of genes involved in Coenzyme Q biosynthesis in Drosophila melanogaster models Coenzyme Q deficiency in humans

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Our work tries to demonstrate that *Drosophila melanogaster* can be used as a model for Coenzyme Q (CoQ) primary deficiency in mitochondrial pathologies during development and adulthood. We used flies with UAS-GAL4 inducible RNAi against the following *COQ* human genes homologues (catalytic and regulatory genes of CoQ biosynthesis pathway): *PDSS1 (CG31005), PDSS2 (CG10585), COQ2 (CG9613), COQ3 (CG9249), COQ5 (CG2453), COQ6 (CG7277), COQ7 (CG14437), COQ8 (CG32649), COQ9 (CG30493), COQ10 (CG9410)*. We cultured the silenced flies at three different temperatures (29, 25 and 18°C) to generate different stress conditions and inductor GAL4 expression levels, and measured COQ genes RNA levels, CoQ deficiency, presence of CoQ biosynthesis pathway intermediaries, development time delays and viability at different development stages (eggs, late larvae, pupae and adult fly) for all temperatures. Our results demonstrate that flies with RNAi against *COQ* genes show a decrease in CoQ levels dependant on the affected gene and intensity of the gene silencing, and we always found stronger disease phenotypes in flies cultured at 29°C (thermical stress and higher GAL4 expression). RNAi against the gene *PDSS2* showed the hardest phenotype with flies arresting their development cycle after egg hatching. RNAi against *PDSS1, COQ2, COQ3, COQ5, COQ7, COQ8, COQ9* and *COQ10* produced letality at larvae or pupae development stages, and when the affected gene was COQ6, flies managed to achieve adult fly stage but suffered from severe CoQ deficiency. We detected the presence of 5-dimethoxy ubiquinol (DMQ), an intermediary from the latest stages of the CoQ biosynthesis, in flies with RNAi against the genes *COQ3, COQ6, COQ7* and *COQ9*.

These results suggest that Drosophila melanogaster flies with RNAi against COQ human homologue genes can be used as reliable models of mitochondrial diseases with primary CoQ deficiency. Our future research will use these flies as models to study mitochondrial biogenesis in the mitochondrial pathology and during embryonic development.

P13-73
Acetylation is increased in KRAS mutated colorectal cancer cells that are resistant to anti-EGFR therapies

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Colorectal cancer (CRC) is a highly prevalent tumor and a major cause of cancer-related death. Newly developed molecular targeted therapies have provided significant benefits in treating CRC, increasing overall survival. However, oncogenic *KRAS* activating mutations in CRC are associated with lack of benefit from anti-EGFR therapies. Ras activation stimulates cell proliferation via MAPK and PI3K pathways. Here we used isogenic human colorectal tumor cell lines that expressed only WT or mutant *KRAS* to investigate the impact of *KRAS* mutation on cell growth and global gene expression. Mutated *KRAS* through

the activation of the phosphorylation cascade would induce epigenetic changes, leading to a deregulation of the cell key functions and development of the carcinogenic process. Cancer cells harboring *KRAS* mutations displayed significantly increased growth rates compared with WT *KRAS* cells. Besidesmutated cells clearly showed resistance to serum depletion, whereas proliferation of the isogenic WT cells was inhibited. As expected, upon EGF stimuli mutated *KRAS* cells had increased rates of p-ERK and this phosphorylation was sustained for longer periods. Gene expression microarrays have been performed to identify those genes that are differentially expressed in both cell lines. Finally, with regard to protein acetylation due to constitutive Kras activation, we have demonstrated that the pattern of acetylated proteins was increased in mutated cells compared with other cell variants in which *KRAS* is in wild type status. We postulate that post-translational modifications other than phosphorylation, such as protein acetylations could play a key role in the modulation of signaling pathways and pharmacological resistance in CRC. Grants: PI 09/02480; ACOMP 12/069 and GentxGent 23/09.

P13-74
MicroRNAs, miR-1, miR-133a/b and miR-208 in infarcted and remote myocardium of human myocardial infarction with the focus on the ventricular fibrilation and/or tachycardia

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MicroRNAs (miRNAs), *miR-1, miR-133a/b*, and *miR-208*, described as muscle or cardiac specific, are involved in post-transcriptional regulation of gene expression in physiological, developmental and pathologic processes in heart. However, knowledge about their role in ventricular fibrillation and/or tachycardia (VF/VT) in patients with myocardial infarction (MI) is limited. The samples from the infarcted and border zones, as well as from the remote myocardium were available from 47 patient with MI, 23 with clinically proven VF/VT, and 24 without VF/VT. The control group consisted of heart tissue samples from 8 trauma victims. The qPCR and statistical analysis were performed to obtain changes in miRNAs expression. In infarcted and remote myocardium (with and without VF/VT) in comparison to control group there was statistical significant down–regulation of *miR-133a* and up-regulation of *miR-208*, and *miR-1* up-regulation in remote myocardium. When comparing infarcted to remote myocardium we found statistical significant down-regulation of *miR-1* in infarcted zone in patients with and without VF/VT, as well as up-regulation of *miR-133a* in infarcted zone of patients without VF/VT. The most important, we found statistical significant down-regulation of *miR-133a* in infarcted zone of patients with MI with VF/VT compared to those without VF/VT. MicroRNA*miR-133a* down-regulation in patients with VF/VT might indicate association of *miR-133a* and development of VF/VT after MI, since HCN2, subunit of the ion channel of the heart pacemaker, is suggested as one of the targets for *miR-133a.* Second, altered expression of some investigated miRNAs was found not only in infarcted zone but also in remote myocardium of patients with MI, suggesting remodelling of remote myocardium after MI.

P13-75
Overexpression of PARP6 is associated with good prognosis in colorectal cancer

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Background: Poly(ADP-ribose) polymerase (PARP) is an enzyme that mediates post-translational modification of proteins. Seventeen known members of the PARP superfamily can be grouped into three classes based on catalytic activity: (i) classical poly(ADP-ribose) polymerases, (ii) mono(ADP-ribosyl) transferases, and (iii) catalytically inactive members. PARP6 belongs to the mono(ADP-ribosyl) transferase class.
Aim: To investigate PARP-6 expression and the clinico-pathological features such as location, Duke's stage, histological pattern and prognosis in colorectal cancer and examine the function of PARP6 in HeLa cell lines.
Results: Immunohistochemical analysis revealed that PARP6 positivity was found at higher frequencies in colorectal cancerous tissues and was inversely correlated with loss of histological differentiation. We also found that in primary colorectal cancer with distant metastasis and with stage D the PARP6-negative cases were significantly higher than the PARP6-positive cases. Furthermore, PARP6 positivity negatively correlated with the Ki-67 proliferation index. Kaplan-Meier analysis showed that PARP6-positive colorectal cancer had a good prognosis. In HeLa cells, the expression of PARP6 induced growth suppression, but a PARP6 mutant with a C-terminal deletion lacking the catalytic domain, had no effect.
Conclusion: PARP6 acts as a tumor suppressor through its role in cell cycle control. PARP-6 expression can be as a potential novel molecular marker of aggressive neoplasia.

P13-76
Oxysterol-induced soluble endoglin release and its involvement in hypertension

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Hypoxia in the placenta is considered as the base of the pathogenesis of preeclampsia, a pregnancy-specific syndrome where soluble endoglin (sEng) has been described as a prognostic marker. We investigated the effects of hypoxia and the downstream pathways in the release of sEng in the trophoblast-like cell line JAR. Under hypoxic conditions, we found an increase in the sEng released to the JAR culture medium parallel to an elevated formation of reactive oxygen species (ROS). Because this augment in the ROS levels is usually related with the formation of oxysterols, we assessed the 22-(R)-hydroxycholesterol (22-R), which has been described as a natural ligand of the liver X receptor (LXR). Treatment of JAR cells with either 22-(R) or the LXR synthetic agonist, T0901317 (T09), resulted in a clear increase of sEng. The involvement of the LXR pathway was corroborated using mouse embryonic fibroblasts (MEFs) derived from a LXR double knock-out mouse transfected with human endoglin. Moreover, human endoglin from stable cell transfectants underwent proteolytic cleavage under hypoxia. Treatment of JAR cells with T09 showed an increased MMP-14 expression and activity as well as

a significant reduction of its endogenous inhibitor TIMP-3 at mRNA and protein levels. The involvement of MMP-14/TIMP-3 pathway in sEng release was confirmed by using TIMP-3 specific siRNA, recombinant TIMP-3 and recombinant MMP-14. Interestingly, endoglin-related peptides carrying the consensus MMP-14 cleavage site G-L blocked the sEng shedding in JAR cells. Also, mice treated with either T09 or 22-R underwent an increase in the plasmatic sEng levels, concomitant with an augment in the systolic arterial pressure (SAP). Similarly, mice overexpressing either MMP-14 or sEng showed increased values of SAP. Finally, administration of an endoglin peptide containing the MMP-14 cleavage site prevented the oxysterol-dependent increase of SAP and sEng levels in mice. These studies provide a clue to understand the involvement of the LXR pathway in the sEng release and its contribution to vascular haemostasis regulation.

P13-77
Coenzyme Q biosynthesis is regulated by RNA-protein interaction

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Coenzyme Q (CoQ) deficiency is a rare disorder with a variable phenotypic presentation that includes pure myopathy, myopathy with encephalopathy, cerebellar atrophy with ataxia, and infantile multisystem disease including encephalopathy and nephrophaty, and nephritic syndrome. Primary CoQ deficiency arises from mutations in COQ genes, while secondary forms of CoQ deficiency are caused by mutations in genes not involved in CoQ biosynthesis. In most patients, the exact site and nature of the defects on biosynthesis have not yet been identified. Because CoQ biosynthesis is complex and not fully defined, identification of the molecular genetic defects has been challenging. At least ten genes (*COQ1-COQ10*) forming a multi-peptide complex are required for CoQ biosynthesis. One of them, *COQ7*, is a central regulator of the pathway. We have previously demonstrated that NF-kB regulates*COQ7* gene transcription under oxidative stress. Our current studies have uncovered the interaction of the RNA-binding protein (RBP) HuR and other as-yet unidentified RBPs with the 3′UTR region of the *COQ7* mRNA. We propose a model of post-transcriptional regulation of COQ7 expression, whereby RBPs binding to the COQ7 3′UTR can rapidly and effectively alter COQ7 expression levels to adapt to changing cellular needs for cellular CoQ activity.

P13-78
Bisphenol A exposure during adulthood alters aromatase and 5α-reductase isozymes expression in prostate of rat

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Background: The high incidence of prostate cancer and benign prostatic hypertrophy in the ederly men is a cause of increasing public health concern. In recent years, various environmental disrupting chemicals, as a Bisphenol A (BPA), have been shown to disrupt sexual and reproductive organs, including the prostate gland. However, the mechanisms underlying these effects remain unclear.
Objetives: Because androgens and estrogens are important factors in the prostate pathophysiology, our objective was to examine, in ventral prostate of rat, the effects of adult exposure to BPA on 5α-reductase (5α-R type 1, 2 and 3) and aromatase, key enzymes in the biosynthesis of dyhidrotestosterone and estradiol, respectively.
Methods: Adult rats were subcutaneously injected with BPA (25, 50, 300 and 600 μg/Kg bw/day) dissolved in vehicle for four days. mRNA and protein levels were analyzed by quantitative RT-PCR and immunohistochemistry respectively.
Results: All BPA-treated groups had lower mRNA and protein levels of both 5α-R1 and 5α-R2 versus controls but higher mRNA levels of 5α-R3, a biomarker of malignancy. Aromatase mRNA and protein levels were also increased by BPA. An increase in this enzyme has been described in prostate diseases. BPA-treated rats also evidenced an increased plasma estradiol/testosterone ratio, which is associated with prostate pathology.
Conclusions: Our results may provide new insights regarding the role of BPA in the development of prostate disease and could be of great value for the study of prostate disease risk associated with exposure to BPA in adulthood.

P13r-79
Dimerization of the dual-specificity phosphatase laforin: identification and mutagenesis of implicated residues

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Laforin, one of the proteins involved in Lafora Disease (LD, OMIM 254780), presents a modular structure composed by a carbohydrate-binding domain and a dual-specificity phosphatase domain. Although its physiological activity as a carbohydrate phosphatase has been proposed, the interaction with malin – an E3 ubiquitin ligase also involved in the disease - has been described. This interaction results in the formation of a functional complex, implicated in the regulation of many other processes unrelated to laforin's phosphatase activity, like proteasomal degradation and autophagy.

It has been described the capability of laforin to form dimers. Laforin monomers were first postulated to be inactive and the dimerization necessary for the phosphatase activity. However, it has been recently described that laforin activity does not depend on dimerization, and that laforin is present mainly as a monomeric form. The function of laforin dimers and their relation with LD remains nuclear, although it has been described that dimerization depends on redox conditions, suggesting that disulphide bonds are involved in the process.

Using directed mutagenesis we have designed laforin forms in which cysteine residues have been replaced by serine. One of these mutants (C329S) was uncapable to form dimers both in bacterial and mammalian cells. Besides, its catalytic activity was drastically impaired, although expression levels of the protein were similar to the wild type.

Taking together, these results suggest that cysteine 329 is specifically involved in the dimerization process of laforin and hence the C329S mutant constitutes a valuable tool in order to analyze the physiological implications of laforin's oligomerization.

P13-80
Genetic testing and rare diseases in Europe: activities of the European Society of Human Genetics

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The European Society of Human Genetics (www.eshg.org; ESHG) is a non-profit, non-governmental organization which has two main aims: (i) to promote research in basic and applied human and medical genetics and (b) to ensure high professional standards in diagnostic and clinical practice. ESHG also facilitates contacts between scientists and professionals who share these aims, in particular those working and/or residing in Europe. ESHG was established in 1967 and is one of the founding members of the International Federation of Human Genetics Societies. ESHG also has several important committees such as the Annual Meetings Committee who selects venues for ESHG conferences (which have over 2000 participants, including an increasing number of exhibitors), the Scientific Programme Committee who prepares attractive scientific programs for these meetings, Educational Committee dealing with pre-graduate and post-graduate education in genetics, or the Public and Professional Policy Committee who issues consensual policy documents and/or position statements on current topics. Activities of its Quality Committee, dealing with coordination of external quality assessment in molecular genetics and cytogenetics stem from the European Commission Network of Excellence project EuroGentest (www.eurogentest.org) that aimed at harmonization and standardization of genetic services in Europe. ESHG works closely with European National Human Genetics Societies. Finally, since 80% of rare diseases are of genetic origin the activities of the Orphanet portal (www.orpha.net) in terms of genetic testing will be presented.

P13-81
Analysis of specific genes expression in intestinal ischemia-reperfusion injury in rats

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The ischemia-reperfusion of the small intestine induces an inflammatory response triggered by tissue injury which involves the action of cytokines and other inflammatory mediators. The accumulation of inflammatory mediators can increase bacterial translocation by damaging junctions between epithelial cells in the mucosa of the small intestine. The delicate balance of the cytokine expression seems to be a key factor which can lead to hyperinflammation or immunosuppression and development of Multiple organ dysfunction syndromes (MODS). The aim of this study was to analyse mRNA expression of specific apoptotic genes (Bcl2, Bax) and cytokines (TNF alpha, TGFB2, IL10, IL6 and IL1beta) in ischemia-reperfusion injury of the small intestine. In the experiment, male Westar rats underwent 1h ischemia that was performed by complete occlusion of mesenteric artery. Samples were harvested after 1 hour, 24 hour and 30 days of reperfusion. Total RNA was isolated from the complete wall of the small intestine and purified. mRNA quantification of specific genes was performed by real-time RT-PCR system Mx3000P using relative standard curve method. All the examined genes showed similar tendency. The quantity of mRNA reached the highest level after 1hour of reperfusion, and then (after 24 hours and 30 days of reperfusion) the mRNA level decreased significantly. After 24 hours of reperfusion, regeneration mechanisms in the small intestine have started. Ischemia-reperfusion injury is a complex and specific process. We can conclude that analysed cytokines are the main mediators with established correlation with trauma, and seem to be the target of research for the future therapeutic alternatives.

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Keywords: ischemia-reperfusion, apoptosis, real-time RT-PCR, cytokines, bax, bcl2, rat.

P13-82
Proteomic investigation of plasma membrane proteins involved in HBV infection

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Hepatitis B virus (HBV) is an enveloped DNA virus member of the *Hepadnaviridae* family. Infection with HBV is a very serious health problem, resulting in acute and chronic hepatitis, cirrhosis and frequently hepatocellular carcinoma. Although there is an efficient vaccine available, more than 350 million people are known to carry the virus around the world. The viral particle is composed of a nucleocapsid containg the partially double-stranded DNA genome surrounded by the viral envelope. Usually viral infection begins with receptor recognition at the host cell plasma membrane, followed by highly specific cell-virus interactions. The early steps of HBV entry in target cells are largely unknown, because of the poor infectivity *in vitro* and the absence of a robust tissue-culture model to support virus infection. In this study we have made use of the HepaRG cells, the only proliferating cell line permissive for HBV infection following a differentiation treatment, to identify proteins with potential role in the HBV life-cycle. Mass spectrometry analysis in differentiated and non-differentiated cells showed an increased expression of several proteins upon differentiation process. Of these targets, 5 proteins where further taken into consideration for validation by Western-Blot and Immunofluorescence. Consistent with the mass spectrometry data, an important up-regulation of 3 proteins was confirmed. Further studies, linking these proteins with the infection process of HBV will be performed.

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P13-83
Inorganic polyphosphate is functionally related with the nucleolus in human myeloma cells

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There has recently been increasing interest in inorganic polyphosphate. This polymer accumulates in platelet granules and its functions include modulating various stages in blood coagulation, inducing angiogenesis and provoking apoptosis of plasma cells. However, the distribution and functions of intracellular polyphosphate in blood cells are poorly understood. In this work, we evaluate the characteristics of polyphosphate in myeloma cells lines and in primary myeloma cells from patients. We have developed a novel method for detecting levels of polyphosphate in cell populations using flow cytometry, and have found that myeloma plasma cells present higher levels of polyphosphate than normal plasma cells and other B cell populations. Mass-spectrometry-based proteomics of polyphosphate-rich subcellular fractions, and confocal microscopy experiments, indicate that polyphosphate accumulates at high levels in the nucleolus of myeloma cells. In addition, the inhibition of the nucleolar transcription produced a drastic modification in the cellular distribution of polyphosphate. RNA polymerase I activity, responsible for transcription in the nucleolus, is also modulated by polyphosphate, in a dose-dependent manner. Together, our results suggest that polyphosphate metabolism could be an attractive target for myeloma treatment, because of both the unusually high levels of the polymer and its functional relationship with the nucleolus.

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P13-84
Sialic acid treatment makes cancer cells distinguishable from normal cells

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In contrast to normal cells, cancer cells have structural changes of surface glycans caused by their glycosylation. The terminal monosaccharide of glycoconjugates on eukaryotic cell surface is typically a sialic acid (N-acetyl-5-neuraminic acid, SA). The increased sialylation is usually related to progression and poor prognosis of most carcinomas.

Lectins are the proteins that bind specific carbohydrate residues on the cell surface. It is well known that plant and fungal lectins have strong antitumor effects. The treatment of cells by SA can change the cell surface glycosylation profile, possibly by amplifying the lectins target sites.

We tried to make the differences between normal (normal mammary epithelial cell line HB4A) and cancer (breast cancer cell line T47D) cells using SA treatment and subsequent interaction with 3 plant lectins: Maackia amurensis agglutinin I (MAL-I), Sambucus nigra agglutinin (SNA) and Triticum vulgaris agglutinin (WGA). The exposure to SA did not affect any cell viability. Normal and cancer cells display sialoglycoconjugates with all lectins. SA treatment promotes display of $\alpha 2 \rightarrow 3$ sialylated glycans structures on the surface of cancer cells rather than normal cells. Using flow cytometry and confocal imaging we have shown that SA treatment resulted enhanced binding with the SA of specific lectins. However, the MAL-I showed a very strong staining on the membrane of sialic acid treated T47D cells. We suppose that using of MAL-I on SA treated cancer cells can stimulate the development of new diagnostic and prognostic biomarkers.

P13-85
Characterization of *COQ4* in the synthesis of coenzyme Q6 in *S. cerevisiae*

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Coenzyme Q is a redox lipid that has important functions in the cell such as the transport of electrons from complex I, and II to complex III in the mitochondrial respiratory chain and its role as antioxidant. At least, nine genes (*COQ1-COQ9*) are involved in the biosynthesis of this molecule, among them *COQ4*. *COQ4* is essential for the synthesis of CoQ in eukaryotes, but until this moment, its exact function remains unknown. Furthermore, this gene has gained more importance in the last years because several cases of patients with CoQ deficiency syndrome associated to mutations in *COQ4* have been described (Salviati et al., 2012). In yeast, Coqs proteins form a multienzymatic complex organised around Coq4p (Marbois et al., 2009), which seems to have no enzymatic activity but plays a structural role in the complex. In order to discover the function of Coq4p in the synthesis of CoQ is necessary to study both its interaction with others Coqs proteins and the exact point in the biosynthetic process in which Coq4p takes part.

Yeast mutants harbouring deletions in *COQ3-COQ9* genes accumulate the same early precursor, hexaprenyl hidroxybenzoate (HHB) (Clarke, 2001). However, it has been reported that a null COQ7 mutant yeast over-expressing *ABC1*/*COQ8* accumulates a CoQ late intermediate, demethoxy-Q_6 (DMQ) (Padilla et al., 2008). This model can be very useful to study the action point of Coq4p in the CoQ biosynthetic pathway.

In order to elucidate the function of Coq4p, we have developed several point mutants in *COQ4* as well as a *COQ4*/*COQ7* double mutant. Based in results obtained from these mutants, we propose that *COQ4* is necessary in the earliest stage in the assembly of de CoQ biosynthetic complex.

P13r-86
Factors that induce tumor progression through metabolic modulation in breast cancer

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Cancer cells have a unique metabolic signature which enables them to grow under limitation of nutrients, energy and oxygen. This shift modulates several pathways, such as macromolecule synthesis, glycolysis, glucose uptake and lipid generation, which are essential for cell growth and survival. Breast cancer, one of the most common cancers among women, is classified into estrogen receptor-α positive (ER+) and estrogen receptor-α negative

(ER−) subtypes. Each group differs in its propensity for recurrence and survival, being the ER− the worst subtype. Our aim is to identify new metabolic related genes that are necessary for breast cancer progression and malignancy. Using gene expression analysis from human mammary epithelial cells, ER+ and ER− tumor cell lines, and clinical patients, we have identified two key transcription factors of the forkhead family that play a major role in the primary tumor progression and differentiation. We have observed that their expression is mutually exclusive and required for the primary tumor growth, both in cell lines and in xenograft models by modulating the same metabolic gene. Additionally, the factors have opposite effects on tumor cell differentiation regulating the luminal and basal commitment respectively. In conclusion, these data evidence the importance of our selected genes in the tumor growth and development, making them, possible targets for cancer therapy.

P13-87
The combined effect of high glucose and TNF-alpha on proteasomal pathway in rat skeletal muscle L6 cell line

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Hypercatabolic syndrome is a biochemical state characterized by increased inflammatory cytokines (e.g. TNF-alpha) and catabolic hormone levels. Diabetes mellitus is a hypercatabolic disease and one of the most important metabolic consequences of it is muscle protein breakdown. In this study, we aimed to create a hypercatabolic state by applying high concentration of both glucose and TNF-alpha to the differentiated skeletal muscle cells.

Rat myoblasts (L6) were grown in DMEM including 10% FBS, and 5 mM glucose. For differentiation into myotubes, the serum content was reduced to 2%. When the cells were differentiated, they were incubated in mediums with different concentrations of glucose (5–50 mM) and TNF-alpha (0–200 ng/ml) for various incubation times. Beta5 and C2 subunits of proteasomes have been analyzed in Western blot and real-time PCR. We also evaluated the combined effect of these molecules on the cells by using the same experimental design. Here, the optimum doses and incubation times were chosen.

As preliminary results; in high glucose treatment, expression of the catalytic subunit (beta5) of proteasome has reached to the highest level at 25 mM glucose and 24 h incubation time. In TNF-alpha treatments, gene and protein expressions of beta5 were significantly increased at 50 ng/ml. These increments were more marked at 12 and 24 h rather than 6 h. When L6 cells incubated with optimum doses of both glucose and TNF-alpha, beta5, beta5i and NFkappaB protein expressions significantly increased according to control cells.

In conclusion, the both components of hypercatabolic syndrome; hyperglycemia and high TNF-alpha levels may explain activated proteasomal pathway which leads to the muscle wasting observed in diabetic patients. According to our results, single and combined usage of them had different effects on proteasomal pathway of L6 cells.

Keywords: hyperglycemia; TNF alpha; proteasome; skeletal muscle cells.

P13-88
Characterization of radiation-induced damage in mice heart tissue

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Radiation induced damage of cardiovascular system is one of reported side effects of radiotherapy. Heart failure related to radiotherapy most possibly involves long-term effects of damage of cardiac microcirculation, yet detailed mechanism of such damage remains unclear. Here we aimed to analyze long-term effects of ionizing radiation upon cardiac cells in mouse system. To characterize changes induced by ionizing radiation we have used mouse heart model. Adult male C57BL/6J mice were irradiated with 0.2, 2, 8, 16Gy doses delivered in one fraction to whole volume of a heart, and sacrificed at different time points after irradiation (12 hour–60 weeks after the treatment). Cardiac fibrosis was analyzed by Gomori Trichrome protocol. Density of microvasculature and level of Hsp70i proteins were compared in control and irradiated heart tissue after immunostaining with anti-CD31 Ab and anti-Hsp70i Ab. Induction of apoptosis in irradiated heart tissue was assessed by TUNEL test. In addition to histochemical studies we analyzed activity of autophagic marker LC3, and changes in blood plasma using MALDI-ToF mass spectrometry. We observed slight increase of collagen fibres in irradiated hearts, and slight decrease in microvessles denstity in exposed dose-dependent manner. Inducible heat shock protein HSP70i was observed in irradiated heart tissue at longer times after the exposure (20–60 weeks). Induction of apoptosis in irradiated heart tissue was observed at short times (12–120 hours) after irradiation, analysis of autophagy did not show autophagic activity. MALDI–ToF analysis also did not show significant changes in blood plasma irradiated and control mice. This work was supported by The Ministry of Science and Higher Education, Grant NN 402 685640 and SP5-Euratom 221403 CARDIORISK.

P13-89
Idiophatic generalized epilepsy and gamma amino butyric acid (GABA) B1 receptor genetic polymorphisms (G1465a and C59T) in Turkish population

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Epilepsy is one of the most common neurological diseases worldwide. Gamma amino butyric acid (GABA), the most important inhibitory neurotransmitter of the central nervous system, and its receptors are commonly mentioned in the pathophysiology of epilepsies. Some of the single nucleotide polymorphisms in the genes encoding GABA receptors have been reported to increase susceptibility to temporal lobe epilepsy. On the other hand, idiopathic epilepsy, which is the most widespread (65%) when etiologically classified, has been less studied in this respect. In Turkish population, genetic polymorphisms of $GABA_{B1}$ receptor have not been studied in relation to IGE before. For these reasons, in this study we aimed to determine the frequencies of two

genetic polymorphisms (G1465A and C59T) of $GABA_{B1}$ receptor in Turkish population by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method, and to investigate the correlation between these polymorphisms and idiopathic generalized epilepsy (IGE). In this context, 196 IGE patients and 109 controls were included in this study.

G1465A polymorphism is located in exon 11 of $GABA_{B1}$ gene and changes glycine to serine (Gly489Ser). There are contradictory findings on the association of this polymorphism and epilepsy in the literature. We did not observe polymorphic alleles (1465A) in Turkish population for $GABA_{B1}$ G1465A in this study. C59T nucleotide exchange causes a substitution of alanine to valine (Ala20Val) in exon1a1. Polymorphic allele (59T) frequencies were found to be 0.102 and 0.110 in IGE patients and controls, respectively ($p = 0.756$). In conclusion, these two variants of $GABA_{B1}$ receptor are not related with idiopathic generalized epilepsy in Turkish population.

Keywords: idiophatic generalized epilepsy, GABA, genetic polymorphism, Turkish population

P13-90
In vitro differential effects of sexual hormones on mitochondrial dynamics and adiponectin expression in white adipocytes

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In vivo studies have demonstrated a sexual dimorphism in mitochondrial function in several rat tissues, as well as in the main markers of insulin resistance. The existence of a link between mitochondrial dysfunction and the impaired insulin sensitivity has been postulated. Adiponectin, an adipokine secreted by white adipose tissue (WAT) is proposed as that link. To further explore the role of sexual hormones in WAT mitochondrial function and adiponectin expression as well as their relationship with insulin sensitivity, an *in vitro* study using a murine preadipocyte cell line (3T3-L1) was performed. Cells were treated either with progesterone, testosterone or estradiol. Markers of mitochondrial function (cytochrome oxidase, citrate synthase) and dynamics (cardiolipin, mitochondrial DNA, mitofusins 1 and 2, optic atrophy 1, mitochondrial fission protein 1 and dynamin-related protein 1), adiponectin and AdipoR1 and AdipoR2 were analyzed. Results show an increase in cardiolipin content with all hormones, whereas mitochondrial DNA levels were only enhanced with estradiol and progesterone treatments. Expression of all the main markers of mitochondrial dynamics, adiponectin, AdipoR1 and AdipoR2 was downregulated by progesterone and testosterone, while estrogens did not produce any significant effect. These results show that sexual hormones modulate both mitochondrial function and adiponectin expression of white adipocytes, so they may be involved in the sexual dimorphism in these processes in response to diverse physiopathological stimuli.

P13-91
Actin isoforms and adhesion junctions reorganization in epithelial–mesenchymal transition of cervical carcinoma cells

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Malignant cellular transformation requires alteration in cell migration. The disruption of actin cytoskeleton and intercellular adhesions is an important component of the acquisition of invasive properties in epithelial malignancies. Carcinoma cells invasive ability is associated with reduced expression of the adhesion junctions' molecules and induced expression of mesenchymal markers, frequently referred as epithelial–mesenchymal transition (EMT). The molecular changes in EMT program include: downregulation of epithelial markers, such as E-cadherin, alpha-catenin and keratins, as well as increased expression of mesenchymal proteins N-cadherin, fibronectin and vimentin. Actin reorganization is also involved in EMT process. We studied the difference in the epithelial and mesenchymal markers' profiles between non-malignant keratinocytes HaCaT and cervical carcinoma cell lines C33A, SiHa and Caski. Epithelial marker E-cadherin reduced expression in the cervical cancer cells was accompanied by the enhancement of mesenchymal proteins N-cadherin and vimentin expression. Downregulated expression of E-cadherin was associated with increased expression of transcriptional factor Snail. Beta-cytoplasmic actin was more abundant in non-malignant keratinocytes, compared to cervical carcinoma cells. We used epidermal growth factor (EGF) and mitochondria-targeted antioxidants to find out how transformation degree affects marker profile and MAPK signaling pathways. EGF upregulated expression of the mesenchymal proteins and activated ERK1/2 signaling pathway. Mitochondria-targeted antioxidants, as well as MEK1/2 inhibitor U0126, prevented EGF-induced transformation development. Antioxidants initiated changes in the cell shape, cytoskeleton organization, E-cadherin-positive intercellular contacts and MAPK signaling in cervical cancer cells.

P13-92
Extracellular matrix peptides upregulate collagen I expression on hepatic stellate cells

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The turnover of extracellular matrix components can generate signals that regulate several cell functions such as proliferation and differentiation. Synthetic peptides with sequences present in extracellular matrix proteins were found to induce both stimulating and apoptotic effects on several cell types including monocytes/macrophages, the main producers of metalloprotease- 9 and other inflammatory mediators, and hepatic stellate cells (HSC) the primary cellular source of matrix components in liver disease. In this work we analyzed some possible effects of two peptides of fibronectin and collagen IV, FN10 and COL27 respectively, on HSC and monocytes/macrophages.

We show these peptides promote collagen I expression in HSC. We also found that both peptides triggered signalling pathways that resulted in increased JNK and p38 activities in these cells. The involvement of these signalling molecules in the fibrogenic process induced by extracellular matrix peptides was confirmed by using specific inhibitors.

In addition, we observed that FN10 and COL27 peptides increased metalloprotease-9 production by monocytes/macrophages generating a positive loop that would contribute to the sustained overproduction of this protease observed in inflammatory liver diseases.

Therefore, extracellular matrix peptides could play an important role in controlling the fibrogenic response in liver pathologies generally associated to inflammatory stress, by regulating monocyte and HSC functions.

P13r-93
Endoglin is involved in the ERK 1/2 signaling pathway

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Endoglin (CD105) is a transforming growth factor-β (TGF-β) transmembrane co-receptor that structurally belongs to the Zona Pellucida (ZP) family of proteins. It is predominantly expressed at the surface of endothelial cells playing an important role in vascular remodeling, homeostasis and pathology. Endoglin has a large highly glycosylated extracellular domain containing the juxtamembrane ZP domain of about 260 amino acids and the orphan domain (OD) that is responsible for ligand binding. Endoglin modulates TGF-β responses mediated by Smad proteins, but little is known about its effect on other signaling routes. Recently, we have reported that endoglin inhibits the extracellular-signal regulated kinase 1/2 (ERK 1/2) phosphorylation in carcinoma cells (Santibanez et al., 2010; Carcinogenesis 31:2145–2154). ERK 1/2 is a serine/threonine protein kinase member of the mitogen activated protein kinase (MAPK) family that phosphorylates Jun and Fos transcription factors of the AP-1 complex. The ERK1/2 cascade regulates many different and opposite cellular processes such as proliferation, differentiation, survival, apoptosis and stress responses. In mitotic cells, an abnormal activation of this pathway contributes to the development of different pathologies, including cancer. Despite the preliminary data supporting a possible interaction between MAPK cascade and endoglin, the molecular basis of this interaction is still poorly understood. Interestingly, we have found that using an AP-1-Luc reporter, a truncated construct containing the endoglin ZP-domain promotes the ERK pathway. Because a soluble form of endoglin that contains the ZP domain is associated with tumor progression and aggressiveness, we put forward a model in which soluble endoglin could modulate this process via interaction with ERK. Currently, our efforts are focused on trying to elucidate the putative interaction between the ZP domain of Endoglin and ERK1/2 pathway.

P13-94
Memory T cells are related with microvascular obstruction in ST segment acute myocardial infarction

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Purpose: In ST segment elevation myocardial infarction (STEMI) restoration of coronary artery flow might result in microvascular obstruction (MVO). The relationship of this process with lymphocyte trafficking has not been fully defined. The aim of this study was to determine the relationship between T memory cells with MVO in reperfused STEMI.
Methods: We studied 30 patients with a first STEMI treated with percutaneous revascularization. Distinct subtypes of memory lymphocytes: T naïve (CD45RACD4), T effector memory (TEM) cells (CD45ROCD4CD62L−), T memory central (CD45RO CD4CD62L+) and chemokine receptors CXCR3 and CCR4 were serially determined by flow cytometry before reperfusion and 24, 96 hour and 30 days afterwards; values were compared with 30 age- and sex-matched control subjects with normal coronary arteries assessed by angiography. Cardiac magnetic resonance was used to detect microvascular obstruction during the first week after the infarction.
Results: In comparison with controls, patients displayed a higher number of circulating TEM cells after infarction. In STEMI patients there was a significant increase of TEM cells during the first 96 hours comparing with values previous to reperfusion ($p < 0.05$) and a recovering of pre-reperfusion levels within next month was observed. An increase of TEM cells was correlated with more MVO ($p < 0.01$) it was remarkable that even before reperfusion TEM cells were increased in those patients with more MVO. An intensification of lymphocyte trafficking after 24 hours was shown with further CXCR3 and CD4CCR4 expression ($p < 0.001$ in both cases).
Conclusions: Lymphocyte trafficking understood as an increase of memory T cells and chemokine receptors expression are closely related to MVO and it could be a pathophysiological mechanism to explain MVO plugging in reperfused myocardium. Further studies will be needed to determine if an increase of TEM cells in the first stages of MI could be a useful predictor of MVO.

P13r-95
Recombinant human carbamyl-phosphate synthetase 1 (CPS1): at last a workbench for understanding the urea cycle disease CPS1 deficiency

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CPS1, a 1500-residue 6-domain enzyme, is the most abundant liver mitochondrial matrix protein. It catalyzes the urea cycle first step. This cycle converts the neurotoxic waste product of protein catabolism, ammonia, into urea. *CPS1* gene mutations can cause CPS1 deficiency, with hyperammonaemic coma and death. >130 missense mutations were reported in CPS1 deficiency, for which experimental evaluation of their disease-causing potential was not possible without a robust system for mutating, expressing and purifying recombinant human CPS1 (hCPS). We now report this system, using baculovirus and insect cells, with mg yields of pure mature hCPS having a N-terminal 6His-tag. This enzyme is indistinguishable kinetically from the enzyme isolated from human liver, and has allowed more in-deep studies on hCPS1 than those existing thus far (based on only two publications on liver-purified hCPS1). Our studies include investigations of the enzyme domain structure, of hCPS stability versus thermal, oxidative stress and proteolytic insults, and on the actions of the substrates and of

the CPS1 essential activator, acetylglutamate, on CPS1 stability. The possibility of using the commercial orphan drug and acetylglutamate analog carbamylglutamate as a chemical chaperone for stabilizing hCPS1 has also been examined with the purpose of opening the way to novel therapies for CPS1 deficiency. The value of this recombinant production system for investigating intra-hCPS signal transduction and for exploring the effects of clinical mutations found in CPS1 deficiency is also demonstrated here with an assorted sample of mutations, including previously reported clinical mutations. Some of these clinical mutations affect kinetic parameters for the substrates, other mutations affect acetylglutamate activation and still others affect stability or folding of the enzyme. Supported by grants from: the Spanish (BFU2011-30407 and SAF2010-17933) and Valencian (Prometeo) Governments; the Alicia Koplowitz Foundation, and NIHU54H-D061221.

P13-96
Novel role of TAR-DNA-binding protein-43 in mitochondria and the regulation of oxidative phosphorylation

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TAR-DNA-binding protein-43 (TDP-43) is one of the main components of cytoplasmic inclusions associated with neurodegenerative disorders [1]. We have recently linked its alteration to cellular stress [2]. Mitochondrial dysfunction is also associated to neurodegenerative disorders [3] and previous studies have shown that mutated forms of proteins involved in neurodegeneration (SOD1, Aβ peptide, huntingtin) induce mitochondrial failure [4]. Therefore, a link between TDP-43 alteration and mitochondrial dysfunction could be hypothesized in neurodegenerative disorders associated with TDP-43 pathology.

To test this hypothesis, we studied the mitochondrial function of Neuro 2A cells over-expressing various truncated forms of the human TDP-43 protein, associated with neurodegeneration.

Over-expression of TDP-43 truncated forms (162-414, 219-414, 274-414) during 24 or 48 hours induced typical cytoplasmic inclusions, without changes in cell viability. Nevertheless, high resolution respirometry revealed a marked decrease in the basal oxygen consumption rate particularly with the smallest TDP-43 transcript (274–414), which is the pronest to aggregation. In line with this, cells over-expressing TDP-43 (274–414) showed a marked loss of mitochondrial respiratory chain complexes I, II, III and IV representative peptides as analyzed by western immunoblot.

Furthermore, immunofluorescence assays demonstrated mitochondrial protein (CoxII) aggregates colocalized with TDP-43 inclusions, overall suggesting a mitochondrial failure. Supporting these results, it was observed an increase of mitofusin-2 (MFN2) levels, while the porin levels remained unchanged. In addition, active levels of the serine protease Omi/HtrA2, checkpoint of the mitochondrial quality control, increased significantly in cells over-expressing truncated forms of TDP-43.

In summary, these results show a novel pathogenic property of TDP-43 aggregates, affecting both oxidative phosphorylation system and mitochondrial function.

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P13-97
Dengue virus capsid protein interacts specifically with very low density lipoproteins

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Dengue virus (DENV) causes 50 million cases of a serious mosquito-borne human disease, leading to more than 20 000 deaths every year. Nowadays, the disease vectors are spreading to North America and Europe, where autochthonous infections were detected in 2010. No specific and effective treatment is currently available, in part due to an incomplete understanding of the viral components interactions with the host cellular structures. Following previous works focused on the DENV capsid (C) protein interaction with human intracellular lipid droplets (LDs) [1,2], we tested the interaction between DENV C and both low and very low density lipoproteins (LDL and VLDL, respectively), by atomic force microscopy (AFM) based force spectroscopy and dynamic light scattering (DLS). Our results revealed a specific DENV C interaction with VLDL, pointing to a molecular target present on VLDL but not on LDL. This interaction is potassium ion dependent, which had identically been observed for the DENV C-LDs interaction [1]. Taking into account the similarities between LDs and VLDL, and among the proteins perilipin 3 (or TIP47; suggested as DENV C target on LDs [1]) and ApoE (present on VLDL but not on LDL), these results indicate ApoE as the DENV C molecular target on VLDL. Our results also suggest that DENV may possibly form lipoviroparticles – a fusion between viral particles and immature VLDL. Such structures represent a great advantage for the replication cycle of other related viruses, such as the hepatitis C virus (HCV). This would constitute a novel step on the DENV replication cycle.

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P13-98
HER2 gene amplification, phosphorylated-HER2, EGFR expression and DNA ploidy in urinary bladder carcinomas

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Spontaneous dimerization of HER2 receptors in tumours with HER2 gene amplification or formation of HER2/EGFR heterodimers leads to receptor autophosphorylation in C-terminal tyrosines which initiates a complex signalling network involved in proliferation, cell invasion and metastasis.

In a series of 28 urinary bladder carcinomas we investigated HER2 gene amplification by chromogenic *in situ* hybridization and HER2, Tyrosine 1248-phosphorylated HER2 (Tyr1248-pHER2) and EGFR expression by immunohistochemistry. DNA content from a single tumour cell suspension was analyzed by flow cytometry for DNA ploidy level.

HER2 overexpression was observed in 14/28 carcinomas and amplification of HER2 gene was present in 6/14 HER2 positive samples. 4/6 cases with HER2 amplification co-expressed Tyr1248-pHER2 and EGFR, were non-diploid and had lymph

node metastases. The expression of Tyr1248-pHER2 and EGFR were higher in non-diploid tumours compared with diploid samples.

The association of HER2, Tyr1248-pHER2 and EGFR overexpression with non-diploid pattern and lymph node metastases, suggests that these proteins are involved in aggressive biological behaviour of urinary bladder carcinomas.

P13-99
Adherence of *Neisseria meningitidis* strains serogroups B, C, W135 and Y to human cells lines: an *in vitro* analysis of menigococcal's infection

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Neisseria meningitidis, a commensal gram-negative bacterium of the human nasopharynx, occasionally cross the respiratory and blood–brain barriers and cause life-threatening diseases such as meningitis and septicemia. Since *N. meningitidis* is a human-specific pathogen, studies with cellular models are an important tool for the analysis of the interaction between this bacteria and its host. The aim of the present work was to analyze 15 *N. meningitidis* strains from serogroups B, C, W135 and Y in the infection of human cells from different sites of meningococcal infection: respiratory tract, endothelium and nervous system. NCIH460 (lung carcinoma), Hec-1B (endometrium adenocarcinoma) and NG97 (glioma) cells were infected and incubated at 37°C under 5% CO_2. After three hours of infection, the samples were inoculated in chocolate agar to determinate the meningococcal adhesion rates, fixed and stained with Wrights' stain to morphological analysis in optical microscopy and had its RNA extracted to determinate the TNF-α, IL-6, lL-8 and IL-10 profile through quantitative Real-time PCR. The morphological alterations such as cell shrinkage and TNF-α expression were more evident in NG97, in which the adhesion rates were between 3% and 58%. The maximum adhesion rates in NCIH460 and Hec-1B were 36% and 57%. The strain B4 stood out with the higher adhesion rates and morphological alterations in Hec-1B and NG97 cells. W135 strains in general were less adherent and injurious. Strains from others serogroups varied among themselves in the evaluated parameters. The data indicate that the meningococcal interaction with host cells is independent of serogroup and the differential adhesion patterns in different cells *in vitro* were capable of mimicking the pathological process in meningococcal infection.

P13-100
Analysis of the splicing factor SRSF1 as a marker for endothelial senescence and its role in vascular pathology

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Aging is the major risk factor *per se* for the development of cardiovascular diseases. The senescence of the endothelial cells (ECs) that line the lumen of blood vessels is the cellular basis for these age-dependent vascular pathologies, including atherosclerosis and hypertension. During their lifespan, ECs may reach a stage of senescence by two different pathways; a replicative one derived from their preprogrammed finite number of cell divisions; and one induced by stress stimuli. Also, certain physiological stimuli, such as transforming growth factor-β, are able to modulate cellular senescence. Currently, the cellular aging process is being widely studied to identify novel molecular markers whose changes correlate with senescence. This analysis focuses on the regulation of alternative splicing mediated by the serine-arginine splicing factor 1 (SRSF1, or ASF/SF2) during endothelial senescence, a process that is associated with a differential subcellular localization of SRSF1, which typically exhibits a scattered distribution throughout the cytoplasm. Based on its senescence-dependent involvement in alternative splicing, we postulate that SRSF1 is a key marker of EC senescence, regulating the expression of alternative isoforms of target genes such as endoglin (*ENG*), vascular endothelial growth factor A (*VEGFA*), tissue factor (*T3*), or lamin A (*LMNA*) that integratle in a common molecular senescence program.

P13r-101
PINK1: an anti-apoptotic and anti-proliferative protein in human breast cancer cells

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Cancer and Parkinson's disease (PD) are two disorders for which the final pathophysiological mechanism is not fully defined. Epidemiologic data indicate that people who develop PD have a decreased risk of cancer with exception of malignant melanoma, and skin, thyroid and breast cancers, suggesting a functional linkage between PD and cancer. In favor of this, misregulation of mitochondria homeostasis is considered an important hallmark in the pathogenesis of both diseases. Recently, several genes associated to PD, including Parkin, LRRK2, DJ-1, and PINK1, have been proposed as modulators of cancer processes. Mutations on PINK1 (PTEN-Induced Kinase 1) gene are associated with hereditary early onset PD. PINK1 is a putative protein kinase whose mRNA is expressed at high levels in several carcinoma cell lines and in PTEN-overexpressing cells. PINK1 protects cells against mitochondrial-mediated apoptosis, controlling mitochondrial homeostasis through electron transport chain, mitochondrial membrane potential, calcium homeostasis and ROS generation. Moreover, PINK1 together with Parkin, has been associated to mitochondrial fission/fusion processes and mitophagy.

To define the putative role of PINK1 in cancer in relation with the mitochondrial homeostasis, we present the analysis of survival/proliferation processes of MCF-7 human breast cancer cells overexpressing PINK1 wt or PINK1 G309D, a mutation linked to PD. Our results demonstrate that PINK1 wt overexpressing cells, but not PINK1 G309D, have an anti-apoptotic role in mitochondrial-mediated apoptosis in breast cancer. Moreover, PINK1 wt, but not PINK1 G309D, inhibits proliferation, invasion, and anchorage-independent growth of breast cancer cells, likely by cell cycle progression inhibition.

P13-102
Atypical case of muscle-eye-brain disease with minimal myopathological changes: clinical features and molecular characterization

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Muscle-eye-brain disease (MEB, MIM 253280) is a congenital muscular dystrophy clinically characterized by muscle weakness and structural defects in the brain and the eye. MEB belongs to a group of rare muscular dystrophies with autosomal recessive inheritance known as dystroglycanopathies that are caused by a deficient O-glycosylation of α-dystroglycan (α-DG), a central component of the dystrophin-glycoprotein complex. MEB is caused almost exclusively by mutations in the gene encoding protein O-mannose beta-1,2-*N*-acetylglucosaminyltransferase 1 (POMGNT1), which catalyzes the second step in the O-mannosylation of α-DG. Here we describe a severe case of MEB with pronounced brain and eye anomalies, but without a clear dystrophic pattern in muscle biopsy, in which no glycosylation of α-DG was detected by both immunohistochemistry and western blotting. Mutation screening revealed two different heterozygous mutations in the POMGNT1 gene: (i) c.1545delC (p.Tyr516Thrfs*21), a novel truncating mutation which results in a loss of the C-terminal 125 amino acids, and (ii) c.1469G > A (p.Cys490Tyr), a previously identified missense mutation. Both mutations affect residues located in the catalytic domain of the POMGNT1 protein, and hence it is tempting to speculate that they are pathogenic. In conclusion, our findings broaden the clinical spectrum of MEB and suggest that mutation analysis of this gene may be useful in patients with severe structural brain and ocular defects, mental retardation and congenital hypotonia, independent of the severity of the dystrophic pattern in skeletal muscle.

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P13-103
Overexpression of the adaptor protein RukI/CIN85 increases the stemness potential of MCF-7 breast adenocarcinoma cells

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Cancer initiating cells (CICs) are a subset of the bulk tumor cells responsible for initiation and progression of oncologic diseases. While a large body of work concerning molecular factors that drive the development of CICs phenotype has been carried out, a number of issues with respect to the role of certain adaptor proteins remain still open. The expression level of SH3-containing adaptor protein Ruk/CIN85 was shown to be increased in a number of human tumors but the exact molecular mechanisms by which Ruk/CIN85 affects carcinogenesis are largely unknown. Therefore, we investigated the stemness potential of Ruk/CIN85 by overexpressing its full-length isoform in weakly invasive MCF-7 breast adenocarcinoma cells. The Ruk_I/CIN85 overexpressing cells showed a slower growth rate, decreased cell adhesion, an enhanced anchorage-independent growth in soft agar, constitutive activation of Src and Akt kinases, elevated level of CD44 marker, and increased resistance to doxorubicin treatment. Stable overexpression of Ruk_I/CIN85 also induced hypoxia inducible factor (HIF)-1alpha protein level and HIF-1 activity under hypoxia as well as increased activity of NF-kappaB transcription factor. Transwell migration and wound healing assays revealed that Ruk_I/CIN85 overexpressing cells possessed increased motility. It was also demonstrated that relatively high percentage of suspension cells as compared to adherent cells excluded Toluidine blue and this ability correlated with the level of Ruk_I/CIN85 expression. When subjected to mammosphere forming conditions, floating MCF-7 cells with Ruk_I/CIN85 overexpression quickly developed mammospheres. Taken together, the data obtained indicate the potential regulatory role of adaptor protein Ruk/CIN85 in the development of CICs-like phenotype in breast adenocarcinoma MCF-7 cells.

P13r-104
A novel inducible model for the study of PTEN-induced carcinogenesis

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PTEN is one of the most frequently mutated tumor supressor genes in human cancers. Monoallelic loss of PTEN has been found in 30–80% of human cancers, including lymphomas, endometrial, thyroid, breast or prostate cancer. As full deletion of the gene shows embryonic lethal phenotype, tissue specific models are needed. We have developed the first PTEN conditional inducible model for study the role of this protein in epithelial tissues. Using the Cre/LoxP technology we deleted PTEN exon 5 from 4 week-old mice by single tamoxifen injection. On reaching 6 weeks after tamoxifen treatment, PTEN deletion was detected in epithelial compartment of different tissues. One hundred percent of animals showed endometrial and prostate cancer, as well as very severe thyroid hyperplasia. Epithelial cells of other organs where PTEN was deleted such as the tubular cells of the kidney, hepatocytes or epithelial lung cells did not develop neoplastic growth. Moreover, we tested the mTOR inhibitor Everolimus (RAD001) in PTEN-induced neoplasias. After 2 weeks of treatment, tumor free survival was dramatically increased and lesions were reduced. Tissues from Everolimus treated animals showed high apoptotic activity and low cell proliferation. This model may provide a useful platform for the study of PTEN-induced epithelial carcinogenesis and drug testing in different organs using a single mouse model, but retaining normal expression in other cell components such as stromal cells.

P13-105
The polyQ protein ataxin-3 protects against stress conditions

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Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disorder caused by the expansion beyond a certain threshold of a polyQ tract in the protein ataxin-3 (AT3). AT3 consists of a globular N-terminal Josephin domain and of a flexible C-terminal tail containing the poly-Q tract. The pathology results from protein misfolding and intracellular accumulation of fibrillar amyloid-like aggregates. The loss of function resulting from misfolding might also be involved in the mechanisms of pathogenesis. AT3 is a conserved and ubiquitous protein known to bind polyubiquitin chains and to function as a deubiquitinating enzyme. It seems to be involved in different cellular pathways, i.e. aggresome formation and ubiquitin-proteasome pathway, but the physiological role is still poorly understood. To investigate possible functions of AT3 in cellular pathways that respond to altered protein homeostasis, we constitutively expressed several AT3 variants in *Pichia pastoris*, a methylotrophic yeast lacking proteins homologous to AT3. Growth assays showed that expression of wild type AT3 or the sole Josephin domain allows yeast growth even under stress conditions, such as heat-shock or ER stresses (dithiothreitol, tunicamycin). The expression of a catalytically inactive variant could not restore yeast viability, which suggests that the deubiquitinating activity of AT3 is required for the protective effect. We also created a transgenic *C. elegans* strain over-expressing wild type AT3 in neurons under the control of a pan-neuronal promoter *unc-119*. Based on body bends counting, preliminary studies suggest that under stress condition *C. elegans* over-expressing wild-type AT3 shows an increased motility compared with wild type. Our data suggest that AT3, thanks to its deubiquitinating activity, displays a protective effect against stress conditions.

P13-106
Allelic frequency of single nucleotide polymorphisms involved in cancer related bioprocesses

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Chronic and complex diseases stem mainly from the complex interactions of genes with the environment. Numerous genetic polymorphisms have been identified, directly or indirectly contribute to susceptibility to complex diseases. Cancer is one of the complex diseases and second causes of deaths in the worldwide. In this study we aim to assess allelic frequency of cancer-related single nucleotide polymorphisms in Turkish participants.

Genotyping was performed using MALDI_TOF based mass spectrometry. The genetic variations predisposing to cancer etiology were analyzed according to manufacturer's protocol of Sequenom hME platform. Results were statistically analyzed for SNPs; MnSOD rs1799725, GSTP1 rs1695, GSTP1 rs1138272, CYP1A1 rs4646903, CYP1B1 rs1800440, CYP1B1 rs1056836, COMT rs4680, CYP17A1 rs743572, ELAC2 rs34152967, CYP19A1 rs10046, SRD5A2 rs523349, SRD5A2 rs9282858.

Genotype and allele frequencies were calculated and compared with European, Asian and African populations and allelic distributions mainly were close to the European community Genotyping results for five SNPs were inconsistent with the Hardy-Weinberg equation. Some genotypes, causing susceptibility to cancer, were found to be frequent in heterozygote state.

Rapid changes in genetics/genomics knowledge are affecting the content of health services, leading to personalized health care. Preventive health care models which also include genetic testing, allows developing individualized nutritional, pharmacological and medical follow-up advice to reduce risk for chronic diseases. The information on the allelic frequencies of genes in a given population is important for research, development and implementation of personalized health care models which may bring targeted preventative healthcare strategies.

Keywords: genetics of complex diseases, cancer predisposition, SNP genotyping

P13-107
Toxicogenomics in the mitochondrial diseases

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Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function: two rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the oxidative phosphorilation system, which is the 'business end' of oxidative metabolism, where ATP is generated. Therefore, mutations in these genes can cause an energy deficit and cause mitochondrial diseases. The effects of mitochondrial disease can be quite varied and the severity of the specific defect may also be great or small. The pathogenic mutations are usually heteroplasmic whereas neutral polymorphisms are homoplasmic. However, there are many exceptions and an increasing awareness of the possible or documented pathogenicity of homoplasmic mutations. In fact, mutations causing LHON are homoplasmic (11778/ND4, 3460/ND1, and 14484/ND6). Similarly, most nonsyndromic forms of deafness are due to homoplasmic mutations, including A1555G in the 12S-rRNA gene. One possible explanation is that these mutations are modulated by other nuclear or mitochondrial variants and/or the adverse effect of environmental causes. We used transmitochondrial cell line models (cybrids) to analyze whether the combination of mtDNA mutations and xenobiotics (environment factors) may trigger the patient's pathology. We built cybrids with pathological mtDNA mutations in osteosarcoma (143B) and another cell line that shares many characteristics of neuronal progenitor cells, teratocarcinoma (NT2). We analyzed their mitochondrial function and their interactions with different xenobiotics (pesticides, antibiotics and organotin compounds).

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P13-108
SH-SY5Y neuroblastoma cell line for the analysis of the role of mitochondrial polymorphisms in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disease caused by the destruction of dopaminergic neurons. Many factors have been linked to the disease such as mutations in nuclear genes (the products of some of them are connected to mitochondria), nuclear gene inactivation involved in the replication and expression of mitochondrial DNA (mtDNA), mtDNA pathogenic mutations and population polymorphisms and, finally, the action of various xenobiotics that cause dysfunction of oxidative phosphorylation (OXPHOS) system after binding mtDNA-encoded OXPHOS polypeptides. Therefore, mtDNA appears to play an important role in PD.

The development of a stable and reliable dopaminergic neuronal cell model is particularly necessary for studying the pathogenesis of PD and developing therapeutic strategies. Many features of the neuroblastoma cell line SH-SY5Y make it very useful as a model of dopaminergic neurons. Here, we detail the biochemical characterization of the OXPHOS function in undifferentiated and differentiated SH-SY5Y cells and the construction of transmitochondrial cell lines using this nuclear background as a previous step in the analysis of PD-related mtDNA polymorphisms.

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P13m-109
Neuregulin reduces glycemia in insulin resistant situations

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Neuregulin is a growth factor that regulates a wide range of cellular processes in different tissues, among them proliferation, differentiation and energy metabolism. In the present study we analyzed neuregulin effect on whole body glucose utilization (glucose tolerance and insulin tolerance tests) in healthy and in insulin resistant situation. *In vivo* administration for 15 min of a recombinant neuregulin-1 isoform, heregulin-beta1 (177–244) (Hrg) (50 ng/g body weight) reduced glycemia in db/db type 2 diabetic animals in response to a glucose tolerance test. In parallel, insulinemia was also reduced upon neuregulin treatment. More moderate neuregulin effects, but along the same lines, were detected in non-diabetic animals. Although assays were developed in fasting conditions, neuregulin did not induce an hypoglycemic shock neither in control nor in diabetic mice. In order to analyze the tissues that responded to neuregulin to regulate glycemia, we examined glucose production under pyruvate administration, in fasting conditions, to account for the effects on the hepatic glucose output. Neuregulin completely abrogated the increase in glycemia upon pyruvate administration in comparison to saline-treated animals. Neuregulin appeared to inhibit hepatic glucose output by significant increases in protein kinase B (PKB) and glycogen synthase kinase 3 (GSK3b) phophorylation which favour the glycogen synthesis rate. These results suggest an important role of neuregulin in the control of glycemia in healthy and insulin resistant situations due in part by modulation of hepatic glucose metabolism.

P13-110
Effects of ovariectomy and estradiol supplementation on oxidative metabolism and redox status of rat cardiac muscle

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We have previously reported the existence of sexual dimorphism in the energy metabolism of several rat tissues, including cardiac muscle. Since estrogens might be involved in the oxidative metabolism and redox status preservation observed in female rats under different physiological situations, the aim of the present study was to investigate the effects of ovariectomy and 17beta-estradiol (E2) supplementation on these two processes. Fourteen week old female Wistar rats were divided into three experimental groups: control, ovariectomized (at 5 weeks of age) and ovariectomized plus E2 supplementation for the last 4 weeks. Serum progesterone, insulin and total antioxidant capacity were measured. Superoxide dismutase (SOD), glutathione peroxidase (GPx), cytochrome oxidase (COX), and citrate synthase (CS) activities were determined in cardiac tissue homogenates. Hydrogen peroxide production and oxygen consumption were measured in permeabilized cardiac myofibers. Ovariectomy decreased cardiac oxygen consumption and COX and GPx activities, while E2 administration restored control values; SOD activity, however, showed the opposite response. In serum, ovariectomy reduced progesterone levels, whereasE2 treatment increased them.

These results suggest that E2 supplementation would enhance cardiac muscle oxidative metabolism in ovariectomized rats, pointing to a role of estrogens in the sexual dimorphism observed in rat cardiac energy metabolism, although the involvement of other factors cannot be ruled out.

P13r-111
Novel mutations in mitochondrial tRNA genes

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Human mtDNA consists of super-coiled, closed circular, double-stranded DNA molecules of approximately 16.5 kb, located in the mitochondrial matrix. There are several copies per mitochondrion, and they are organized into nucleoids. Human mtDNA encodes 13 essential protein components of the oxidative phosphorylation system, as well as part of translational machinery:

two mt-rRNAs (12S and 16S rRNAs) and 22 mt-tRNAs (MTT) required to synthesize these proteins.

Mitochondrial dysfunction can therefore arise from either nuclear or mitochondrial mutations, and despite accounting for just 5–10% of the mtDNA, pathogenic point-mutations in the MTT genes are responsible for the majority of mitochondrial diseases.

We report, after whole mtDNA sequencing, three new homoplasmic mtDNA mutations in the $tRNA^{Trp}$ (MT-TW), $tRNA^{Val}$ (MT-TV) and $tRNA^{Gly}$ (MT-TG) genes associated with different phenotypes and two new case of mutation previously reported, one of them in the $tRNA^{Ala}$ (MT-TA) gene in a case presenting a MELAS phenotype, and other in the $tRNA^{Trp}$ (MT-TW) gene in a case presenting a Leigh phenotype. We analyzed the pathogenic criteria for these point mutations: evolutionary conservation of the base, presence of heteroplasmy, segregation of the mutation with disease and absent in healthy controls. Moreover, we generated transmitochondrial cybrids with mtDNA of patients and their controls in two different nuclear background, human osteosarcoma line (143B) and human teratocarcinoma line Ntera2 (NT2), in order to determine the pathogenicity of these novel mutations in mitochondrial tRNA genes.

This study underlines the importance of sequencing the entire mtDNA in patients with mitochondrial disease in which the common mutations have been discarded.

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P13-112
Next-generation sequencing facilitates quantitative analysis of psoriasis transcriptome

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Psoriasis is a chronic immune-mediated disease that affects 1–3% of the population. The pathogenic processes, leading to the development of the disease, are complex and involve different signaling cascades.In our research in order to identify differentially expressed genes and molecular alternations associated with psoriasis we have used new perspective high-throughput data analysis tool – RNA-sequencing. We have performed the analysis of four biopsy pairs taken from lesional and non-lesional skin evaluating gene expression changes, non-gene expression, and splicing alternations. Among 20 000 of analyzed genes the expression of 580 varied significantly (p-value ≤ 0.05). Among the most up-regulated genes are *SERPINB4, S100A9, IL8, S100A8, PI3, SERPINB3, TCN1, TMPRSS11D, S100A12, HEPHL1* (log. fold change 2.3–1.69). Most down-regulated genes are *AADCL3, CYP2W1, SEC14L6, ELOVL3, WFDC3, FADS2, PRR15L,PM20D1, IL37, LCE5A* (log. fold change -1.6 to -1.1). Most reinforced cell processes are immune and defense response, response to cytokines, immune cells chemotaxis, cell adhesion, ECM and cytoskeleton remodeling. The most weakened processes are glutathione, lipid, and fatty acid metabolism, response to hormones, and chemical homeostasis. Further investigations must be required for deeper understanding of the molecular basis of the disease.This project could provide a comprehensive understanding of molecular mechanisms involved in the pathogenesis of human immune-mediated diseases such as psoriasis. The combination of new algorithms with high-throughput data will form the basis for novel clinical developments in personalized medicine and deeper understanding of the functioning of immune system.The work was supported by RAS program ''Fundamental Sciences To Medicine''.

P13r-113
TNPO3 binds the HIV-1 assembled capsid and assists HIV-1 replication after nuclear import but prior to integration

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TNPO3 is a nuclear import receptor required for HIV-1 infection. This work explores different aspects on the ability of TNPO3 to assist HIV-1 replication. Depletion of TNPO3 expression dramatically decreases the infection of the primate lentiviruses HIV-1, HIV-2 and SIVmac. We showed that depletion of TNPO3 led to an HIV-1 block after nuclear import but prior to integration. To mechanistically investigate the requirement of TNPO3 in HIV-1 infection, we tested the binding of TNPO3 to the HIV-1 core. We found that TNPO3 binds the HIV-1 core; however, depletion of TNPO3 moderately but consistently affected the normal uncoating process of HIV-1 core. Interestingly, TNPO3 binds a core formed by the capsid mutant N74D with decreased affinity when compared to wild type HIV-1. Because TNPO3 is a nuclear import receptor, we tested the possibility that depletion of TNPO3 might be affecting HIV-1 replication by blocking the nuclear import of a specific factor. We observed that depletion of TNPO3 did not change the subcellular localization of the integration cofactor lens epithelium-derived growth factor (LEDGF/p75) and the cleavage and polyadenylation specific factor 6 (CPSF6). Overall this work suggests that TNPO3 interacts with the incoming HIV-1 core in the cytoplasm to assist a process that is important for HIV-1 infection after nuclear import.

P13-114
Cardiotrophin 1 is a new molecular player in the mechanisms that control energy balance

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Cardiotrophin-1 (CT-1) is a member of the gp130 family of cytokines with important cytoprotective activities. Recent research in obesity has focused on the gp130 receptor ligands as potential therapeutics agents for obesity with conflicting results. We have analyzed the metabolic effects of CT-1 and we showed that this cytokine is a nutritional-regulated gene that is up-regulated by fasting and down-regulated by refeeding. We observed that *ct-1*$^{-/-}$ mice develop mature-onset obesity and insulin resistance despite reduced calorie intake. Decreased energy expenditure preceded and accompanied the development of obesity. Interestingly, administration of recombinant CT-1 (rCT-1) to mice decreased blood glucose in an insulin-independent manner and increased insulin-stimulated AKT phosphorylation in muscle. *In vitro* studies were performed with adipocytes and L6E9 myocytes to analyze glucose uptake. We demonstrated that rCT-1 promoted fatty acid oxidation in muscle through AMPKα2. Chronic rCT-1 treatment reduced food intake, enhanced energy expenditure and induced white adipose tissue remodelling characterized by upregulation of genes implicated in the control of lipolysis, fatty acid oxidation, mitochondrial biogenesis and genes typifying brown fat phenotype. Moreover, rCT-1 reduced body weight and corrected insulin resistance in *ob*/*ob* and in high-fat fed obese mice. Finally, our studies in humans showed that obese subjects had elevated CT-1 serum levels compared to lean subjects and decreased after weight loss, suggesting that the high circulating

levels of CT-1 could be due to an overproduction by enlarged adipose tissue. We conclude that CT-1 is a master regulator of fat and glucose metabolism with potential applications for treatment of obesity and insulin resistance.

P13-115
Melatonin modulates the levels of pro-inflammatory cytokines IFN-γ, TNF-α and IL-17 in peripheral lymphocytes of multiple sclerosis patients

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Melatonin is a compound produced in the pineal gland and in other tissues and cells (as T lymphocytes) that has showed modulatory effects over the immune system and, specially, on the production of mediators like cytokines.

Multiple sclerosis (MS), the most common inflammatory demyelinating disease in adults, is thought to be an autoimmune disease in which autoreactive T cells have a main role, with a complex network of cytokines involved (among them, IFN-γ, TNF-α and IL-17).

The objective of our work is to study the effect of melatonin on the production of IFN-γ, TNF-α and IL-17 in PBMC from MS patients, compared with healthy controls.

PBMC from 20 MS patients and 20 healthy controls were isolated and cultured in presence of melatonin (100 μM) or DMSO (vehicle), and stimulated with PHA or anti CD3/CD28 for 48 hours. Cell viability (WST-1) and proliferation (BrdU) and supernatant levels of IFN-γ, TNF-α and IL-17 were measured. Moreover, intracelular staining for flow cytometry was performed on brefeldin A-treated cells, using anti-CD4, anti-IFN-γ, anti-TNF-α and anti-IL-17 antibodies.

Melatonin significatively reduced number of CD4+ IFN-γ+ cells in non-stimulated and stimulated cells from both controls and patients, and also caused a decrease in CD4+ TNF-α+ and CD4+ IL-17 + cells in stimulated cultures from controls and patients. IFN-γ levels were diminished in stimulated control and patient cells treated with melatonin.

Cell viability and proliferation were not changed in melatonin-treated cells from controls or patients, either stimulated or non-stimulated.

In conclusion, melatonin showed an inhibitory effect in the levels of IFN-γ, TNF-α and IL-17 produced by PBMC of multiple sclerosis patients, and those effects were not caused by diminished cell viability or proliferation.

P13-116
A novel role for BMP9 as a protumorigenic factor in liver

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TGF-β family members play a relevant role in tumorigenic processes, including the hepatocellular carcinoma (HCC), but the specific implication of the BMPs subfamily is still unknown. BMPs are multifunctional signals that have been described to play a central role during development and they are also key in maintaining the homeostasis in adult organisms. Interestingly, dysregulation of BMP signalling can have pathophysiological consequences. Although originally isolated from fetal liver, little is known about BMP9, a BMP family member, and its role in liver. Our results show that BMP9 is a proliferative factor in HCC cell lines, but not in immortalized adult human hepatocytes. In HepG2 cells, BMP9 triggers the canonical pathway, which regulates expression of the Id1 gene. Importantly, we demonstrated by using chemical inhibitors, ligand trap and genetic silencing approaches that HepG2 cells have an autocrine BMP9 production that support their proliferation and their anchorage independent growth. Additionally, a more detailed analysis of the BMP9 effects in HepG2 cells revealed a remarkable BMP9-mediated survival effect against serum starvation-triggered apoptosis. Finally, BMP9 expression was increased in 40% of human HCC tissues compared with healthy liver as revealed by immunohistochemistry analysis, suggesting that BMP9 signaling may be relevant during HCC pathogenesis *in vivo*. All together, our findings provide new clues for a better understanding of BMP contribution in the pathogenesis of HCC that may result in the development of effective and targeted therapeutic interventions.

P13-117
Direct-to-consumer genetic testing

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Since a few years an increasing number of companies are offering personal genome tests directly to consumers via the internet. Most tests are based on genome scans and predict risks of numerous multifactorial diseases such as type 2 diabetes, age-related macular degeneration and heart attack. The tests are offered to empower consumers to take preventive measures and make healthier lifestyle choices, but the predictive ability of the test is subject to debate. In this presentation, I will present several examples of tests that are available via the internet and give insight in their predictive ability and utility.

P13-118
Characterization of the MAPK/Mnk/eIF4E signaling pathway in several breast cancer cell lines

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Eukaryotic initiation factor (eIF4E) binds the 5′ cap structure of the mRNA and recruits the eIF4F complex to promote translation. In the nucleus, eIF4E promotes the export of a subset of mRNAs, several of them involved in cancer progression. In addition, eIF4E is often overexpressed in human cancers and associated with worse clinical outcome and decreased survival. The activity of eIF4E is regulated for two different mechanisms: availability or phosphorylation/dephosphorylation of eIF4E to participate in the initiation step. Several recent studies suggest that the increased phosphorylation of eIF4E at serine 209 may be a key factor in the tumoral progression. The MAP kinases interacting-kinases 1 and 2 (Mnk1/2), responsible of the phosphorylation of the eIF4E, are substrates of both ERK1/2 and p38MAP kinases. We have identified a new isoform of Mnk1, named Mnk1b, which has constitutive eIF4E kinase activity, independent of the activation of the MAP kinase pathways and, moreover, is capable of entering and to remain into the nucleus. With the aim of characterizing the MAPK/MNK/eIF4E signaling pathway in breast cancer, three cell lines (MCF7, MDA-MB-231 and BT474) representing the major molecular subtypes of breast cancer were used in this study (luminal-A; basal-like and luminal-B, respectively). In these cell lines we have determined eIF4E phosphorylation status and eIF4E, Mnk1a and Mnk1b levels. In addition, the effect of several MAPK/Mnks inhibitors or/and the overexpression of Mnk1a/b on proliferation and survival were analyzed by MTT and colony formation assays. Our results showed that MDA-MB-231 cell line is the most sensitive to Mnk inhibitor and presents the lowest Mnk1a levels. Moreover, the overexpression of the isoform Mnk1b seems to increase the viability of these cells. On the other hand, we have observed that, after a high number of passages, BT474 cells showed a decrease in the Mnk1 levels in parallel to an increase in susceptibility to Mnk inhibitor. Altogether our findings indicate that Mnk1a/b plays an important role in the proliferation and survival in breast cancer cell lines. (Funding: SAF2010-21663).

P13-119
Molecular basis of clinically relevant mutations in human GALT: searching for new therapeutic approaches in classical galactosemia

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Herein we describe the functional evaluation of clinically relevant mutations in human GALT, seeking to establish a molecular basis for the phenotypes assigned to galactosemic patients and ultimately to develop new therapeutic approaches.

Classical Galactosemia (CG) is an inherited metabolic disorder caused by deficient activity of galactose-1-phosphate uridylyltransferase (GALT), resulting from mutations in the *GALT* gene. To date, more than 200 mutations have been described, the majority being missense, which often generate misfolded proteins.

The most frequent mutations described in CG, along with mutations found exclusively in Portuguese patients, were analyzed by expression of WT and mutant enzyme in bacteria.

To characterize the WT and mutant forms, *GALT* WT cDNA was cloned into pET24b(+), mutations were generated by site directed mutagenesis, and expressed in *E. coli* BL21(DE3) Rosetta cells, in minimal medium supplemented with iron and zinc. After purification, comparative functional studies were employed to evaluate the cofactor loading, oligomeric profiles, conformational stability and enzymatic activity of WT versus mutant GALT.

With the perspective of developing chaperone-based therapies for missense mutant GALT, functional rescue was investigated by expressing WT and mutant GALT in an *E. coli* Δ*galT* strain, and comparing the ability of each protein to alleviate galactose toxicity, in the presence and absence of small molecules. Western Blots were performed to evaluate the effect of media supplementation on the soluble protein levels for each recombinant GALT variant.

The results confirmed the disease-causing phenotypes for the selected mutations, thus contributing for a better understanding of the mutational *spectrum* in galactosemic patients. Moreover, small molecule supplementation appears to functionally rescue a subset of GALT mutations.

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P13r-120
Activated leptin, insulin receptor and translation signaling in placenta from pregnant women with gestational diabetes mellitus

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Gestational diabetes patients have high plasma levels of insulin and leptin. Placentas from gestational diabetes suffer from structural and functional changes including overgrowth. We have recently found that leptin stimulates protein synthesis in placenta, in a similar way to insulin, by activating protein signaling machinery. We aimed to study the expression of leptin and leptin receptor (LEPR), as well as LEPR and insulin receptor (IR) activation. Thus, we investigated the phosphorylation of downstream proteins of both the LEPR and IR, including the initiation of translation signaling. We also checked the protein synthesis rate.

We have studied ten control placentas and ten placentas from patients with GDM. Leptin and LEPR expression were determined by quantitative real time-PCR and immunoblot. Protein phosphorylation was measured by specific immunoblot. The rate of protein synthesis was assessed by [3H] leucine incorporation experiments. We have found that leptin and LEPR expression are increased in placentas from GDM, and both LEPR and IR are activated as well as the signaling from both receptors in placentas from GDM compared with placentas controls. Finally, the translation machinery activity and protein synthesis rate were also higher in placentas from GDM. In conclusion, we have found for the first time the activation of leptin and insulin receptors in placenta from GDM and this activity may contribute to the increased protein synthesis rate that we have found. Nevertheless, the specific contribution of each receptor in the increased protein synthesis rate remains to be investigated.

P13-121
How can we assure the quality of genetic tests? The importance of validation, EQA, and accreditation

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The role of clinical genetic testing laboratories is to produce accurate and timely test results. Data from external quality assessment schemes reveal that errors potentially leading to misdiagnosis are common, often concerning 1–5% of results. Errors of all types are observed: misidentification; false-positive and false-negative genotyping errors; misclassified mutations; serious errors of risk calculation, interpretation and of nomenclature. While the frequency of errors may be similar to other medical laboratory fields, the effects of an error in a genetic test can be significantly greater than in other fields because the tests are typically performed once in a lifetime and the results concern not only the tested individual but also untested relatives and current and future offspring. Consequently a genetic testing error can become fixed in the medical record of the patient and of other family members.

Quality assurance in the genetic testing laboratories has many components, both technical and organisational. Key elements include: Personnel need to be made and shown to be competent before performing clinical testing. Validation, one of the fundamental differences between clinical and research labs, refers to the process used to confirm 'through the provision of objective evidence' that a test performs as required before using it to test patients; the largely qualitative nature of many genetic tests presents a challenge. *IQC* helps the lab to assure that tests meet the defined specification from run to run. *EQA* provides interlaboratory comparison to assure test accuracy, while evaluating reporting and providing continuous education.

The ISO 15189 accreditation standard 'Medical laboratories – Particular requirements for quality and competence' covers all these aspects and more, and is considered as the single most effective route to comprehensive laboratory quality assurance. EuroGentest actively supports and encourages accreditation to ISO 15189, and the number of genetics labs so accredited is increasing rapidly in Europe.

P13-122
Role of AMP kinase in renal disease in cystinosis

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Infantile cystinosis, is characterized by reduced reabsorption of solutes by the renal proximal tubule (RPT), due to an autosomal recessive mutation in cystinosin, a lysosomal cysteine transporter. To determine whether reduced solute reabsorption is due to altered transporters as well as apoptosis, cystinosis has been induced in primary cultures of rabbit RPT cells using siRNA against cystinosin.The resulting reduction in Na/Pi cotransport was reduced was associated with a reduction apical NaPi2a transporters, rather than the Na gradient. Nevertheless, intracellular ATP levels were 50% lower in primary RPT cells with a cystinosin knockdown. Further studies indicated that ADP was elevated, and AMP Kinase (AMPK) activated in primary RPT cells with a cystinosin knockdown. In addition, the primary RPT cells with a cystinosin knockdown exhibited increased apoptosis in response to TNFα and cisplatin. The hypothesis was examined that AMPK activation was responsible for the increased apoptosis. Compound C (an AMPK inhibitor) was employed, as well as siRNA against the AMPKα subunit (whose phosphorylation leads to AMPK activation). Compound C prevented the increased sensitivity to toxicants in primary RPT cells with a cystinosin knockdown. However, no significant effect was observed when knocking down AMPKα1 (the predominant α subunit in kidney). Thus, further studies were conducted knocking down both AMPKα1 and AMPKα2. Under these latter conditions, increased cisplatin toxicity was no longer observed in primary RPT cell with a cystinosin knockdown. Experiments are in progress to determine whether activation of AMPKα2 is solely responsible for the increased sensitivity to cisplatin, or whether both α1 and α2 are involved.

P13-123
coq7 (*cg14437*) interference courses a primary coenzyme Q deficiency in *Drosophila*

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The syndrome of coenzyme Q (CoQ) deficiency is a heterogeneous mitochondrial disease characterized by a diminution of CoQ content that affects all the electron transport processes of which CoQ is responsible, like the electron transference in mitochondria for ATP production and the antioxidant capacity that it exerts in all the cell membranes and lipoproteins. Since mutations in either of the genes involved in the synthesis of CoQ course lethality during development, we studied the phenotype using the UAS-GAL4 system to allow the expression of RNAi to interfere one of these genes using a ubiquitous driver (*daughterless*-GAL4).

Within this work we described the interference of *coq7* (*cg14437*) gene, which encoded for a mitochondrial di-iron hydroxylase that catalyzes the penultimate reaction for CoQ biosynthesis. The interference affected the level of *coq7* mRNA and so the catalytic activity that it exerts, coursing a severe reduction in the CoQ content at all the developmental stages and an accumulation of 5-dimethoxy ubiquinone (DMQ), the intermediary compound in the CoQ biosynthesis that is the substrate of the COQ7 protein. Since *Drosophila* presented three species of CoQ (Q8, Q9 and Q10), the coq7 interference drives to the accumulation of DMQ intermediaries for all of them.

The mutant phenotype included pupal lethality at 25°C and around 20% of survival lowering the temperature to 18°C. Eclosed flies presented developmental delay, reduced fertility, short life span and higher ROS production during aging. Mitochondrial preparations showed low oxygen consumption and deficit in the electron transference.

These results demonstrated that coq7 interference in *Drosophila* melanogaster can be used as a reliable model of human mitochondrial diseases with Coenzyme Q deficiency, allowing further studies on mitochondrial biogenesis during the development of a mitochondrial pathology, but also in the therapy against neurodegeneration.

P13-124
Fibroblast to myofibroblast transition – crucial step in asthma progression

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Fibroblast to myofibroblast transition represents a crucial step in the connective tissue deformations typical for fibrocontractive diseases. TGF-β-induced- *de novo* expression of α-smooth muscle actin (α-SMA) and its incorporation into stress fibres conferring high contractile activity to myofibroblasts are basic hallmarks of myodifferentiation. However, several studies revealed the role of crosstalk between and Wnt signalling(canonical TGF-β/Smad pathway in this process. We have demonstrated that human bronchial fibroblasts (HBFs) from asthmatic patients displayed a trait, which facilitated their differentiation into myofibroblasts in culture. HBFs obtained from non-asthmatic subjects and exposed to TGF-β showed a moderate fibroblast-to-myofibroblast transition (FMT), in contrast to abundant myofibroblast fraction derived from HBFs of asthmatics. Experiments on HBFs derived from asthmatic and healthy subjects revealed a difference in reactivity between these cells, upon stimulation with TGF and lithium chloride – an unspecific activator of(−β Wnt signalling. In contrast to control cells, in asthmatics FMT was decreased in the presence of TGF-β/Li, as measured by the percentage of α-SMA positive cells. A similar effect was observed when HBFs were cultured in the presence of TGF-β (and(TWS 119 – a specific inhibitor of GSK-3β and activator of Wnt signalling. (It is intriguing, that a cross-talk between Wnt and TGF-β signaling pathways may result in opposite effects, depending on biological context. Possibly, understanding these differences could help in the development of new therapeutic strategies against asthma.

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P13-125
Nuclear Factor of Activated T cells (NFAT) as a switch inducing a pro-angiogenic integrin profile in endothelial cells

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Nuclear Factor of Activated T cells (NFAT) is a good candidate to provide a switching mechanism that decides whether pro-angiogenic or pro-inflammatory program should be taken in during activation of endothelial cells. NFAT is known to be involved in angiogenesis by up regulation of Cox-2 activity, it is stimulated by VEGF, while its downregulation inhibits angiogenesis. In this report we attempted to identify integrin receptors that may be controlled by NFAT in response to VEGF, and thus involved in this process. Human Umbilical Vein Endothelial Cells (HUVECs) were treated either with VEGF165 or TNFα and expression of integrin subunits (α2, α4, α5, α6, αV, β1,β3, β4 and β5) was evaluated at the mRNA level (RT-PCR), total protein level (Western immunoblotting) and at the surface of the cells (Flow Cytometry).

Our data showed that there was a differential effect of the pro-angiogenic and pro-inflammatory stimulators on integrin expression profiles in HUVECs. TNF upregulated both, the αV subunit and intact αVβ3 receptor, while integrin subunits α6, β4, β5 and intact αVβ5 were downregulated. In contrast, VEGF selectively increased expression of α2, α4, α5, α6, β1, β4, and β5 as well as intact receptors such as α2β1 and α5β1. Of integrin subunits, alteration of the β4 expression seems to be unique indicating that it is a strongly inducible and very sensitive marker of endothelial cells proangiogenic activation. All the observed changes in integrin expression were abolished to different extent by NFAT inhibitors (cyclosporin A, 11R-VIVIT) as well as siRNA specific to NFATc1 and NFATc2. The most consistent inhibition was observed in the case of α6 (pro-angiogenic) and αV (pro-inflammatory and also pro-angiogenic) integrin subunits.

In conclusion, our data support the role of NFAT in inducing either pro-angiogenic or pro-inflammatory state of endothelial cells by expression modulation of different sets of integrin receptors.

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P13-126
Determination of HER2 gene amplification by chromogenic in situ hybridization (CISH) in breast carcinoma

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Detection of Her2neu amplification is an integral part of breast carcinoma diagnostics to decide therapy. The aim of the study was to demonstrate that chromogenic *in situ* hybridisation (CISH), is a useful technique to determine *Her-2/neu* oncogene status in breast carcinoma.

Material and methods: We study 55 cases of breast carcinoma embended paraffin tissues, both CISH and FISH were performed on each case using (SPoT-Light® HER2) for CISH and both Her-2 and chromosome 17 probes for FISH (Vysis). Sixty tumor cells were evaluated in each case. The scoring system and interpretation of CISH were as follows: (i) no amplification (< 5 brown dots/nucleus), (ii) amplification (> 10 brown dots/nucleus), and (iii) low-level amplification (5–9 brown dots/nucleus).

Concordance between CISH and FISH was found in 94.8% cases, considering FISH as gold standard, sensitivity of CISH was 97.5% and specificity 94%.

Cish was introduced as an alternative to FISH, because is more practical alternative due to lower cost, no requirement of fluorescence microscope, use of existing bright-field microscopy and techniques it's similar to IHC, archivable and quantitative results, it s easy to observe both the tissue morphology and the gene amplification evaluation.

Of the 55 cases analyzed, 53 showed similar results for both methods. Two cases were discordant. In these cases, low-level amplification was suggested by CISH but nonamplification by FISH. One of the cases can be explained by polysomy for chromosome 17 by FISH.

In conclusion, our results, suggest that CISH is a useful technique to determine *Her-2/neu*oncogene status, in breast carcinoma for paraffin embedded tissues, is a highly accurate, reproducible and practical technique, with a high sensitivity.

P14 – Molecular Biotechnology

P14-1
Self-bioremediation of cork-proccessing wastewaters by chloro-phenol degrading bacteria immobilised onto residual cork particles

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Cork manufacturing is a traditional industry in Southern Europe; 90% of the world production is concentrated in Portugal and Spain. Cork is a natural product mainly utilised in the production of wine stoppers and as insulation (thermal and acoustic) material. Cork processing begins at boiling the raw material. As a consequence, great volumes of dark wastewaters, with elevated concentrations of chlorophenols, are generated, which must be depurated by costly physicochemical techniques (ozonation, Fenton oxidation, ultrafiltration, adsorption, etc.) before discarding them into public water courses. Bioremediation is an alternative to these expensive physicochemical treatments, having the additional advantage of being applied '*in situ*'.

This work explores the potential of indigenous bacteria, isolated from cork-boiling waters storage ponds, in bioremediation of the same effluent. The bacterial population present in cork-processing wastewaters was analysed by DGGE; low bacterial biodiversity was found. Aerobic bacteria were isolated and investigated for their tolerance against phenol and two chlorophenols. The most tolerant strains were identified by sequencing 16S rDNA. The phenol-degrading capacity was investigated by determining enzyme activities of the phenol-degrading pathway. Moreover, the capacity to form biofilms was analysed in a microtitre plate assay. Finally, the capacity to form biofilms onto the surface of small residual cork particles was evaluated by acridine staining followed by epifluorescence microscopy and by SEM. A low-cost bioremediation system, using phenol-degrading bacteria immobilised onto residual cork particles (a by-product of the industry) is proposed for the remediation of this industrial effluent (self-bioremediation).

P14-2
Towards a magic bullet against malaria: Paul Ehrlich revisited

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Paul Ehrlich's dream of a magic bullet that would seek out and specifically destroy invading microbes is a major aspect of clinical medicine. At present, administration methods of antimalarial drugs release the free compound in the blood stream, thus reducing its availability for *Plasmodium*-infected red blood cells (pRBCs), whereas unspecific drug delivery often demands low concentrations to minimize undesirable side-effects, thus incurring the risk of sublethal doses favouring the appearance of resistant pathogen strains. Targeted nanovector systems can fulfill the objective of achieving the intake of total doses sufficiently low to be innocuous for the patient but that locally are high enough to be lethal for the malaria parasite.

We work on the development of antimalarial drug-carrying nanovectors specifically targeted to pRBCs. Our first immunoliposomal prototype delivers its contents exclusively to pRBCs containing the *P. falciparum* late forms trophozoites and schizonts, improving on average tenfold the efficacy of the antimalarial drug chloroquine. Using chloroquine concentrations well below its IC50, and by modifying parameters such as liposome size, density of targeting antibodies on the liposome surface, and targeted antigen, we approach 100% of parasitemia reduction both *in vitro* and *in vivo* using a murine model for *P. falciparum* malaria. We will discuss our current work aiming at the improvement of the nanovector design through modification of (i) the targeting element, (ii) the encapsulated drug(s), and (iii) the type of nanocapsule, making special emphasis on polymeric structures. Our objective in the short term is the engineering of a nanostructure adequate to enter the preclinical pipeline as an economically affordable new antimalarial therapy.

P14r-3
Terminal protein-primed amplification of heterologous DNA with a minimal replication system based on phage Φ29

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The DNA amplification performed by terminal protein-primed replication systems has not yet been developed for its general use to produce *in vitro* high amounts of DNA linked to terminal protein (TP). Here we present a method to amplify *in vitro* heterologous DNAs using the Φ29 DNA replication machinery and producing DNA with TP covalently attached to the 5′ end. The amplification requires four Φ29 proteins, DNA polymerase, TP, single-stranded DNA binding protein (SSB) and double-stranded DNA binding protein (p6). The DNA to be amplified is inserted between two sequences that are the Φ29 DNA replication origins, consisting of 191 and 194 bp from the left and right ends of the phage genome, respectively. The replication origins do not need to have TP covalently attached beforehand to be functional in amplification and they can be joined to the DNA to be amplified by cloning or ligation. The facts that two functional origins were required at the ends of a linear template DNA and that the kinetics of DNA synthesis was very similar to that obtained using the TP-containing Φ29 genome as template support the proposal that genuine amplification is taking place. Amplification factors of 30-fold have been obtained. Possible applications of DNAs produced by this method are discussed.

P14-4
A peptide tag forming a spontaneous covalent bond applied to study forces at the mammalian cell surface

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The use of peptide tags is a common tool in the spectrum of research in the life sciences; ranging from purification in protein chemistry, immobilization in biotechnology, and identification of different targets in cell biology. However, all of these methods suffer from the same limitations of peptide tags, in that there is low thermodynamic stability compounded by weak mechanical interactions between tag and recognition domain. To overcome these issues, we developed a peptide tag that can form a spontaneous covalent bond between a Lys and Asp residues. This was accomplished by splitting and rationally engineering a domain from the fibronectin binding protein (FbaB) from the infamous 'flesh eating' bacterium *Streptococcus pyogenes*. The process yielded a small 13 amino acid tag (SpyTag) and a 15 kDa protein partner (SpyCatcher). The system was shown to be simple in that it only requires the mixing of the two components for the reaction to occur. It was fast, occurring within minutes and at high yield. In addition the system was robust working in an array of reaction conditions, able to withstand 1 nN of pulling force, flexible in terms of tag location, and functioned *in vivo* as well. With SpyCatcher-SpyTag characterized, we are now investigating its use for cell biological applications, exploring the difference in cell behaviors when protein interactions are covalent. In particular we have used SpyTag to investigate the response of the plasma membrane to force and the stability of cell-cell interactions.

P14m-5
Use of immobilized *Thermotoga maritima* beta-galactosidase for the generation of lactose-free products and galacto-oligosaccharides

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β-galactosidases are biotechnologically relevant because of their use in food industry. They mediate the production of lactose-free products and galacto-oligosacharides (GOS) synthesis, useful as probiotics in functional food formulation. In the current work were used two approaches to improve the proprieties of a recombinant β-galactosidase (TmLac) from *Thermotoga maritima*. First, TmLac was covalently immobilized on epoxy activated magnetic beads and physico-chemical properties were evaluated. Immobilized enzyme showed around 40% higher initial activity than free enzyme. During long-term incubations the performance of both enzymatic forms for hydrolysis and transglicosilation was similar. The immobilized enzyme showed higher stability than free enzyme, at higher and lower temperatures, and it could be re-used during four cycles hydrolyzing completely 5% lactose. Second, TmLac variants were generated through site-directed mutagenesis. From the four mutations (N574S, F571Y, F571W y W962F) only one mutant (N574S) improved the GOS production, while the other three showed a similar (F571Y) or lower (F571W, W959F) GOS production than the wild type enzyme.

P14-6
Which hydrogenases are responsible for H_2 production by *Escherichia coli* during mixed carbon fermentation?

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Escherichia coli possesses four [Ni-Fe] hydrogenases (Hyd) having different role in H2 production and oxidation during glucose or glycerol fermentation.

H_2 evolving activity in the wild type and *hybC* (Hyd-2) mutant grown on mixed carbon (glucose and glycerol) at pH 7.5 was shown to have no changes in the assays with supplemented glucose, compared with those grown on glucose. H_2 production was decreased ~6.5 and ~7.9 fold in *fhlA* (Hyd-3) and *fhlA hyfG* (Hyd-3 Hyd-4) mutants, respectively, compared to the wild type. H_2 evolving activity lowered ~1.8 and ~5 fold in *fhlA* and *fhlA hyfG*, correspondingly, grown on glucose. In *hyaB hybC* (Hyd-1 Hyd-2) mutant grown on mixed carbon H2 production decreased ~1.2 fold compared to the wild type. In contrast, for cells grown on glucose, it was ~1.3 fold higher than in the wild type. For mixed carbon, H_2 production increased ~1.2-fold in *hyaB* mutant compared to the wild type. It was suggested that Hyd-3 with Hyd-4 have H_2 production activity whereas Hyd-1 together with Hyd-2 became responsible for H_2 oxidation when cells grown on mixed carbon.

H_2 evolving activity in wild type grown on mixed carbon was low in the assays with added glycerol, in comparison with that grown on glycerol. This activity was absent when cells were grown on glucose only showing that in the presence of glucose, glycerol can also be fermented. H_2 production activity data obtained for the mutants grown on mixed carbon pointed out the similar role of Hyd-3 and Hyd-4 when glucose was supplemented.

P14-7
The significant role of RNA cap-structure in the assembly of artificial viral particles *in vitro*

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Potato virus X (PVX) is a helical filamentous plant potexvirus. PVX has the possibility of reversible dissociation of virions into coat proteins (CP) and RNA followed by the *in vitro* self-assembly of viral particles. As a result, the structure and biological activity of the virus can be reconstructed.

Previously we showed that incubation *in vitro* of PVX CP with the heterologous RNAs of a number of the various plant and animal viruses results in the artificial viral particles formation. The helical structure and morphology of 'mixed' particles were identical to viral particles assembled from homological PVX RNA and PVX CP and to native PVX virions. It was found that foreign capped RNAs within the artificial viral particles are not available for ribosomes. Our data suggest that the assembly of the 'mixed' particles *in vitro* starts at the 5′-proximal region of the RNA and does not require a specific RNA nucleotide sequence.

To investigate the possible assembly signals we constructed a panel of uncapped 5′ end transcripts of different length and sequence obtained from full genome cDNA of PVX and another potexvirus – Althernantera mosaic virus (AltMV) RNAs. The

possibility of artificial viral particles formation *in vitro* from PVX CP and these transcripts were examinated using the transmission electron and atomic force microscopy. It has been shown that assembly didn't occur. PVX and AltMV RNA transcripts were capped and incubated with PVX CP. As a result the particles morphologically identical to the homological and 'mixed' viral particles were formed. It can be assumed that capping affects the conformational changes in 5′ end transcripts secondary structure and promote its efficient interaction with PVX CP *in vitro*.

P14-8
Evaluation of genetic diversity of hatchery-reared fish used as broodstock in Volta Grande Environmental Station (Minas Gerais, Brazil)

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Captive breeding is used for fish conservation and now, more than ever, for the restoration of declining natural populations. For this, genetic studies may assist important decisions to be taken in areas where human activity has led to changes in river habitat. The aim of this study is to describe the use of DNA markers to evaluate the genetic diversity of the species *Prochilodus lineatus*, used as broodstock in Volta Grande Environmental Station (EAVG). *P. lineatus* is a migratory fish species, endemic in South America and of great commercial, social and environmental importance. A total of 340 broodstocks used in the reproduction of the 2010/2011 season have been micro chipped and had caudal fin tissue sample removed for DNA extraction. After the extraction the DNA was precipitated and the material stored and labeled properly. A DNA database has been made with the isolated DNAs. This genetic bank will allow future genetic analysis such as the historical knowledge of the genetic diversity of the broodstocks used in the EAVG. To investigate the genetic diversity we used DNA markers previously isolated by our group. PCR amplification was made using sets off primers for each DNA molecular marker. Consensus DNA sequence obtained by bidirectional sequencing were aligned and the phylogenetic inference (Neighbor-Joining method) among the samples performed. Results indicated the presence of three different clusters grouping into clades and will contribute to a rational management and choose of new broodstocks to prevent the loss of genetic diversity. In conclusion the isolated molecular markers have showed great potential for studies of population genetics and will contribute with the management of the broodstock and juveniles that will be used in captive breeding as a form of restoration for these species by Volta Grande Environmental Station.

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Keywords: molecular markers, broodstock, *Prochilodus lineatus*, genetic diversity, hatchery-reared fish

P14r-9
Hydrogen photoproduction in *Chlamydomonas*

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In the last years microalgae cultures are receiving special attention due to their capability to produce several compounds that could be used as biofuels; ethanol, methane, triacylglycerols and hydrogen are among the most encouraging ones. Special attention can be paid to hydrogen because (i) the combustion of this carbon-free compound produces only water as product what avoids many environmental problems and (ii) its energetic yield is similar to fossil fuels.

The unicellular green alga Chlamydomonas is widely used to study hydrogen production. Under anaerobic conditions this alga produces a hydrogenase enzyme able to synthesize molecular hydrogen using the energy from either the photosynthetic electron chain or the fermentative degradation of some metabolites (e.g. starch). Unfortunately, hydrogen production in Chlamydomonas is a short, transient process limited by many environmental factors among which the strong inhibition of hydrogenases by oxygen is probably the most limiting one.

Our aim is to understand the influence of the photosynthetic activity and the starch metabolism on hydrogen production. A screening of an insertional mutant library was performed to identify mutants affected in each of these processes. Selected mutants were assessed for photosynthetic parameters, starch accumulation, and hydrogen production. Mutants with increased hydrogen production rates are being analyzed to identify the disrupted gene/s responsible of their phenotypes.

This work focuses in two independent mutants affected in the same locus. Interestingly, these two mutants are able to produce higher rates of hydrogen, relative to parental strain, even under oxygenic conditions.

There are three genes affected in both mutants that may be responsible of the phenotype: a *N*-acetyltransferase (type GCN5), an RNA binding protein, and a SEC24 like protein. We are currently carrying out complementation of both mutants with these three genes in order to determine which gene is responsible of the phenotype.

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P14m-10
Biosynthesis of RNA polymerase inhibitor streptolydigin, pathway regulation and generation of novel derivatives

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Actinomycetes, in particular genus *Streptomyces*, are prolific producers of bioactive compounds, many of them in use for pharmacological or agricultural purposes. However, there is a need for the discovery of novel bioactive compounds to deal with emerging diseases or to improve the properties of drugs in use. This task can be accomplished modifying the structure of known compounds by combinatorial biosynthesis.

Combinatorial biosynthesis involves the isolation and characterization of natural products biosynthesis gene clusters to unravel their biosynthetic pathway. This can be achieved by gene inactivation and gene expression. Once we understand how the compound is synthesized we can afford the rational design of novel derivatives using specific mutant strains previously generated together with the expression of heterologous genes involved in the biosynthesis of other natural products previously characterized.

One of the drawbacks in the generation of novel compounds is the low production normally obtained. This problem can be addressed by understanding how the natural product biosynthesis is regulated. Both regulatory and structural genes can be used to

improve the production of the novel derivatives by over-expressing those genes.

This philosophy to generate novel compounds has been recently used to obtain derivatives of streptolydigin, an acyltetramic antibiotic inhibitor of bacterial RNA polymerase, produced by Streptomyces lydicus and discovered in 1955. The biosynthesis of this compound proceeds through the activity of a hybrid PKS-NRPS system and the attachment of an L-rhodinose moiety. We have determined the biosynthetic pathway of this antibiotic and how its production is regulated. This work led to produce several novel derivatives and new natural products.

P14-11
Site-saturation mutagenesis of formate dehydrogense from *Candida methylica* to increase the thermostability

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Production of optically pure compounds is important for product quality and customer safety especially in pharmaceutical industry. In the synthesis of chirally pure products, enzymatic reactions catalyzed by oxidoreductases are highly sterospecific and very beneficial. However, use of these enzymes is still limited because of the requirement for stoichiometric amounts of the very expensive NAD(P)H coenzyme. NAD^+-dependent formate dehydrogenase (EC 1.2.1.2, FDH) is the last enzyme of methanol metabolism in methylotrophs and is the best and widely used enzyme for regenerating NADH in enzymatic synthesis of optically active compounds. Nevertheless, the lack of thermostability is a disadvantage of native FDH from *Candida methylica* yeast (*cm*FDH). Hence it is important to improve the thermostability of FDH to cope with the harsh conditions in industrial synthsesis procesess. According to the results shown the first residue of *cm*FDH is related to temperature stability (unpublished data), site saturation mutagenesis was applied to the M1 residue. After the generation of mutant library, mutants were selected and screened in terms of thermostability. Results showed that eight mutations at this position are tolerated. If the K_m values are compared individully, except for Met1Arg and Met1Leu, all mutants (Met1Cys, Met1Gln, Met 1Gly, Met 1Asn, Met 1Ala, Met 1Val, Met 1Ser) have better affinity for the substrate formate than that of native *cm*FDH. On the other hand, while Met1Leu has a higher K_m value than that of native *cm*FDH for formate, it has also the best k_{cat}value among the site saturation mutants and the $T_{0.5}$ value of this mutant is almost the same as native *cm*FDH. Further thermodynamic studies are in progress.

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Keywords: formate dehydrogenase, site saturation mutagenesis, thermostability

P14r-12
BEad surface display for affinity screening of protein and peptide binders

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BEad Surface Display (BESD) allows the quantitative analysis and screening of large libraries of protein binders and it is based on water-in-oil emulsions. Single DNA molecules are compartmentalised in droplets with streptavidin-coated magnetic beads and amplified via emulsion PCR. Up to 10^5 copies of beads-bound DNA could be quantified from amplicons of 350–2750 bp. After *in vitro* expression, genotype and phenotype are linked via a SNAP tag and stably coupled to the bead. According to the degree of stringency required by each specific target, display levels can be adjusted between 10^3 and 10^6 copies/bead. The binding properties are analysed by fluorescence activated cell sorting (FACS) and the genotypes of the library members showing the highest affinity recovered individually. When different libraries of the HA tag have been used to test the system, the top 0.3–1% of the population (as analysed by FACS) mostly contained the wt sequence, indicating that BESD allows for the identification of the best binder out of libraries of 10^4 clones after one single round of screening. BESD shares characteristics of both *in vitro* and *in vivo* display systems: being totally *in vitro*, it allows the screening of large size libraries, the possibility to select under non-physiological conditions, the introduction of unnatural amino acids and (bio)chemical modifications. Moreover, it has the advantages of multivalent bacterial and yeast display systems, as it allows the quantitative screening of the whole library and affinity characterisation of individual variants.

P14-13
Biofilm formation and resistence to ethanol of oenological lactic acid bacteria

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Lactic acid bacteria (LAB) are responsible of wine malolactic fermentation, which is a requisite for premium red wines as it positively contributes to aroma and taste complexity as well as to wine microbiological stability. By contrast, uncontrolled LAB growth is responsible of a number of wine alterations, and thus, microbiological control is a key factor for premium wine elaboration. Among bacterial responses to external factors, biofilm formation has been reported as a resistance mechanism to environmental stress (1). The aim of this study was to perform a comparative study of the resistance of oenological LAB of the species *Oenococcus oeni*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* to the presence of ethanol in the culture broth. A total of 69 LAB strains isolated from grapes, musts and wines were analysed. Strains were incubated in the presence of 0%, 2%, 4%, 6%, 8% and 12% (v/v) ethanol in the culture broth; growth rates and cell populations were determined by colony counting. Results showed that all the studied strains were able to grow as planktonic cells in the presence of up to 6% ethanol, reaching maximal cell populations of 10^7–10^9 CFU/ml, and all of them maintained their initial cell populations during 1–2 days incubation in the presence of 12% ethanol, which reveals their resistance to ethanol. Strains were incubated in BHI broth for 24 hour without agitation and biofilm formation was analysed using crystal violet staining method (2) with modifications. Four strains (5.6% of total strains) were demonstrated to be able to form biofilms. These biofilm-forming strains retained their ability to aggregate in presence of up to 6% ethanol, nevertheless, the higher ethanol concentrations, the lesser biofilm formation; and this capacity disappeared in the interval 6–12% ethanol. These results are of relevance for selection of robust LAB starters for biotechnological applications, as well as for improved methods of bacterial disinfection.

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P14-14
Transcriptome analysis shows activation of the arginine deiminase pathway in *lactococcus lactis* as a short-term adaptation to ethanol stress

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Lactic acid bacteria (LAB) are widely used in food biotechnology, and efficient control of LAB growth requires an increase in our knowledge of bacterial behaviour under stress conditions. Ethanol is regarded as one of the most potent inhibitors of microbial growth, and bacterial response to ethanol is an issue of great interest from the point of view of the beverage industry and for our understanding of the molecular basis of bacterial adaptation and survival responses to stress conditions. The aim of this work was to carry out a study of the response of *Lactococcus lactis* to ethanol. *Lactococcus lactis subsp. cremoris* strain NZ9700 was cultivated in GM17 broth with or without 2% ethanol and sampled at mid-exponential growth phase and at the stationary phase. Whole-genome transcriptome analysis was performed using DNA microarrays. Differential expression was observed in 140 genes, among which the highest up-regulation was detected for those involved in arginine degradation through the arginine deiminase (ADI) pathway (20-40 fold up-regulation). In *L. lactis* the ADI pathway is regulated by two transcriptional regulators: ArgR and AhrC, and expression of both regulators is required for complete repression of arginine biosynthesis, while only AhrC is responsible for the activation of arginine catabolism. The metabolic response of wild type *L. lactis* strains was compared to those of the two single deletion regulator mutants MGdelta *arg*R and MGdelta *ahr*C, and the double regulator mutant MGdelta *arg*R*ahr*C. Wild type and mutant *L. lactis* strains were cultivated in absence and in presence of 2% ethanol in a chemically defined medium with arginine (2.5 mg/ml) and without arginine. Results showed that those strains with an active ADI pathway after 10 hour incubation reached higher growth rates and higher cell populations in presence of 2% ethanol when arginine was available, than ADI defective mutants. These results demonstrate the relevance of arginine catabolism for *L. lactis* survival in the presence of ethanol, and are of significance for both food and beverage industries.

P14-15
Clues on protein amyloidosis from RepA-WH1, a synthetic bacterial prionoid

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The complexity of the processes leading to the development of amyloid proteinopathies is counterbalanced by their universal structural basis: the amyloid crossed β-sheets [1]. This has fuelled the quest for disease-unrelated model systems suitable to study protein amyloidosis under quasi-physiological conditions *in vitro* and in simpler organisms *in vivo*. We had reported that engineering RepA-WH1, a bacterial DNA-toggled protein conformational switch (dWH1- > mWH1) functional in plasmid replication [2], enables DNA-modulated control on protein amyloidogenesis *in vitro* [3-4]. We have recently found that RepA-WH1 also interacts *in vitro* with lipid vesicles of defined composition, leading to protein aggregation. RepA-WH1 has thus analogies with nucleic acids and lipid-promoted replication of the mammalian prion (PrP^{C}- > PrP^{Sc}) [5]. When fused to a red fluorescent protein, RepA-WH1 causes an amyloid proteinopathy in *Escherichia coli* which prevented cell proliferation [6]. RepA-WH1 aggregates are not infectious (*horizontally transmissible*), althoug enable conformational templating by cross-seeding *in vitro* [6]. It is thus the first bacterial prionoid [7]. We have recently found that DnaK, the Hsp70 chaperone in *E. coli*, contributes to RepA-WH1 amyloidogenesis *in vivo*, but the Hsp104 chaperone ClpB does not have a major effect on the vertical spread of the amyloid aggregates. Chimaeral RepA-WH1/Sup35 prions are functional in *Saccharomyces cerevisiae* but, driven by the bacterial amyloid sequence, become independent on Hsp104 for propagation.

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P14-16
The novel type of candidate vaccine against influenza virus A based on spherical particles – structural remodeling tobacco mosaic virus

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The new type of nanoplatform based on structural remodeling tobacco mosaic virus (TMV) was obtained and described recently. It was demonstrated that upon thermal denaturation a rod-like TMV transform to spherical particles (SPs). SPs are stable, highly immunogenic, biodegradable and do not contain nucleic acid [1]. The surface of SPs has a unique adsorption capacity. Thereby SPs is a new type of biogenic nanoplatform for presentation of antigens and potentially attractive for vaccine development [2]. Plant viruses are safe for humans, because plants and animals have no common pathogens. Thus plant viruses could be useful platforms for obtaining safe vaccines against different pathogens.

The aim of the present work was the *in vitro* assembly of the novel type of candidate vaccine against influenza virus A based on SPs and receptor binding domain of H1N1-A/California/07/09 hemagglutinin (HA). The formation of SPs-HA compositions was revealed by method of indirect immunofluorescence staining. The immunogenicity of SPs-HA complexes was examined by subcutaneous and intraperitoneal immunization of mice. In both cases of immunizations the titers of serum antibodies to HA increased dramatically when antigen was linked to SPs surface.

Therefore, the model of the highly immunogenic novel candidate vaccine against influenza virus A was developed. The novel technology excludes the possibility of reversion to the pathogenic form and the presence of components that may cause a strong allergic reaction. This approach is safe, simple and could decrease

the cost of vaccine against influenza virus and reduce the period of production.

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P14-17
Cloning, overexpression and characterization of Neu5Ac aldolase from *Lactobacillus sakei* 23 K

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Neu5Ac aldolase (NAL) catalyzes the cleavage and synthesis of Neu5Ac, with an equilibrium that favors cleavage. *Lactobacillus sakei* 23K encodes a NAL putative gene (Uniprot code: Q38V37) (LsNAL). This gene, when cloned and expressed in *Escherichia coli* Rosetta 2 (DE3) pLysS, produced a fully functional enzyme. The recombinant clone with the highest expression was induced with 1.5 mM IPTG in a 5-l fermented at 30°C for 12 hour with vigorous stirring and oxygenation. LsNAL was purified from *E. coli* cells by a two-step procedure, consisting of a 100-kDa ultrafiltration step and Ni^{+2}-chelating affinity chromatography onto a HisTrap FF column, with a 51% yield. The activity of LaNAL was pH-dependent, being the optimum pH of the enzyme for hydrolytic reaction around pH 6, and for synthetic reaction around pH 7. The optimal temperature was also different for both reactions, being 10°C higher for the synthetic reaction (40°C). Thermal stability studies were also carried, finding a melting temperature of 58°C, under the best conditions.

The kinetic parameters were also determined with a K_M of 0.32 mM and a k_{cat}/K_M of 2.8/mM/sec, respectively, for Neu5Ac cleavage. On the synthetic side, the values obtained were of 272 and 12.5 mM and 0.016/mM/sec and 0.31/mM/sec for ManNAc and pyruvate, respectively.

Finally, the *in silico* analysis of its structure and the phylogenetic tree of its sequence classified LsNAL as NAL belonging to group 3.

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P14m-18
CiPCR: a novel restriction-enzyme-, ligase- and recombinase less, autocloning PCR methodology which allows the direct insertion of dsDNA fragments at convenience into circular dsDNA vectors

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Here we describe a novel dsDNA cloning technique based totally in PCR named cloning inverse PCR or CiPCR. This method constitutes a simple and universal technique for cloning dsDNA fragments directly into dsDNA vectors which avoids the standard steps involving restriction enzymes, ligases, topoisomerases, recombinases, and/or especial λ red or equivalent recombination competent *E. coli* cells. CiPCR requires only two PCR steps *in vitro* before the introduction of the constructions in *E. coli* cells. The first step is for the amplification of the insert and it can be done by classical PCR or other amplification methods using CiPCR primers. CiPCR primers contain two sequences linked together, the 3′ half sequences are classical PCR primers to amplify the DNA to be cloned, and the 5′ sequences are the ones that will hybridyze with the desirable sequences in the vector of interest. The blunt-end dsDNA fragments to be cloned, obtained with these two primers, carry 3′ and 5′ end sequences complementary to two vector regions, and are used directly to amplify the vector sequence of interest through these two hybridization signals per each of the two pairs of DNA fragment/vector strands. The hybridization through 3′ end sequence primes the 5′ region of the correspondent template strand of the vector to initiate elongation. The hybridization through the 5′ end sequence with the 3′ region of the same template strand of the vector acts as a polymerase stop signal and defines the original features of CiPCR: (i) delimitation of the exact size of the CiPCR ds amplicon since the first cicle, (ii) CiPCR ds amplicon can not act as template, and (iii) CiPCR amplicons are long-protruding-complementary-end linked-insert-vector dsDNAs able to circularize *in situ* to yield stable vector-type genetic constructions *in vitro* that, once introduced inside *E. coli* cells, became directly cloned and inheritable replicons.

P14-19
Expression, purification and characterization of a macrodomain from *Oceanobacillus iheyensis* HTE-831

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Macrodomains (MacroD) are evolutionarily conserved structural domains found in proteins with diverse cellular functions, which bind modules of NAD^+ metabolites, including ADP-ribose/poly(ADP-ribose) and *O*-acetyl-ADP-ribose (OAADPr). OA-ADPr is produced in reactions catalyzed by NAD^+-dependent protein deacetylases (sirtuins), which regulate gene silencing, metabolic enzymes, life span, and many other cellular processes. OA-ADPr has been implicated as a signaling molecule, modulating several cellular processes.

Computational analysis reveals the presence of a macrodomain-like protein from Oceanobacillus iheyensis HTE-831. In the present poster, the cloning, expression and activity of a recombinant macrodomain from *O. iheyensis* HTE-831 (OiMacroD) is described. It was cloned from gDNA into a different pET vectors, being pET28a where maximal expression was obtained at 20°C and 1 mM IPTG. An efficient two-step purification method was also developed, with a high yield. The molecular mass of purified OiMacroD was determined by SDS-PAGE (22 kDa), by gel filtration (22.6 kDa) and by HPLC/ESI/ion trap (22.59 kDa), confirming the monomeric nature of OiMacroD. The enzyme was active towards OAADPr, rendering ADP-ribose (ADPr) as a product. Thermal stability was also carried out by a thermal melt assay using SyPro Orange, finding that ammonium sulfate and ADPr were the best stabilizers.

A comparative analysis at the level of sequence and homology modelling of OiMacroD and other predicted macrodomains

found in the databases was carried out in order to shed light on the classification of those enzymes.

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P14-20
Comparison of biochemical and immunochemical properties of the recombinant scFv and Fab fragments of antibodies TnIMAB specific to human cardiac troponin I

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Cardiac isoform of human troponin I (hcTnI) is a reliable biomarker of the cardiomyocyte damage which accompanies such severe cardiovascular diseases as myocardial infarction. Monoclonal antibodies TnIMAB are able to recognize hcTnI with high affinity. Usage of recombinant antibodies and their fragments allows improving of existing immunodiagnostic systems and, therefore, is of a high value in fundamental and applied fields of modern biomedicine. Because of the technical difficulties of the whole molecule recombinant antibodies production their fragments are generally used for the implementation of different fundamental and applied tasks. Fab and scFv fragments are the most widespread among them. Fab fragments are characterized by higher affinity and stability. scFv fragments have an advantage of being of small size and having a single chain. In this study, we produced TnIMAB scFv and Fab fragments, compared their production yield, biochemical and immunochemical properties to each other and with the whole molecule antibodies. Light chain and heavy chain gene regions corresponding to TnIMAB scFv and Fab fragments were subcloned pET23a+ plasmids. Expressed fragments were found in the insoluble fraction and were subjected to renaturation followed by affinity chromatography (2–5 mg per 1L of cell suspension). TnIMAB affinity (K_D) measured by surface plasmon resonance approach approximates 3 nM and was not significantly different from recombinant fragments and enzymatic Fab fragments. According to our studies recombinant Fab and scFv fragments specific to hcTnI possess immunochemical activity reaching in some applications the level of whole molecule antibodies and demonstrate all necessary immunochemical properties for being used in diagnostic systems.

P14-21
Identification of the steroid catabolic genes and other genes involved in the complete assimilation of steroids in *Pseudomonas* sp. DOC21

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Pseudomonas sp. DOC21 is a Gram-negative, strictly aerobic, rod-shaped bacterium isolated from soil samples in base to their ability for growing in minimal medium supplied with bile salts (deoxycholate, cholate, lithocholate…), testosterone or androstanolone as the sole carbon source. For the identification of the genes needed to grow in these steroids, a Tn*5* insertional mutagenesis protocol has been performed. By using this technique, 53 transconjugant strains affected in steroid metabolism have been obtained. All these mutants have been classified into two different groups in base to their different behavior when bile salts or testosterone are sole carbon source: the first group include those mutants unable to grow in media containing bile salts as the sole carbon source, although they maintain the ability for growing at the same rate than wild type strain in media supplied with testosterone, androstanolone or 4-androstene-3,17-dione; the other group brings together those mutants handicapped in the use of any steroid as the sole carbon source. By sequencing the adjacent DNA to the Tn*5* insertion points in the different mutants, the genomic region where the genes encoding the steroid catabolic functions clustered has been characterized. According to the classification of the mutants afore mentioned, genes for the catabolism of the lateral chain of bile salts, and ortholog genes to the described for cholesterol (in *Mycobacterium* and *Rhodococcus*) or testosterone (in *Comamonas*) catabolism for steroid rings degradation have been identified. Moreover, we have identified different genes belonging to the cell central metabolism (genes encoding activities from methylcitrate cycle and glyoxylate shunt) that are essential for growing using steroid compounds in *Pseudomonas* sp. DOC21.

P14-22
Uptake of histamine by *Pseudomonas putida* U

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The study of the transport system involved in the catabolism of $^{3}{}_{1}$H-histamine has been approached in *Pseudomonas putida* U. Kinetic measurements were performed *in vivo*, at 30°C in 50 mM phosphate/citrate buffer (pH 6.5). We showed that in this bacterium histamine was taken-up by using at least two different systems: (i) a constitutive one (HCS) which was responsible of the uptake of this amine when *P. putida* U was grown in media containing succinate/glucose but not histamine as the carbon source and (ii) an inducible system (HIS) which appeared when this strain was grown in media containing histamine. The biochemical characterization of both systems revealed that whereas HCS was inhibited by some amino acids (arginine, lysine, glutamine), HIS is stimulated by all them. Furthermore, amines such as bencyse amino acids, Amines as benci imidazole acetic, intermediate of histamine) was diferent.plus histaminelamine, dopamine, 1-phenylethylamine, β-phenylethylamine and tyramine strongly inhibited the HIS but they did not affect HCS.

Moreover, HIS was strongly inhibited by uncouplers of oxidative phosphorylation (2,4-dinitrophenol, sodium azide and carbonyl cyanide 3-chlorophenylhydrazone) as well as by the metabolic inhibitors KCN and sodium arsenate, suggesting that, as expected, it is an active uptake system. The thiol-containing and thiol-modifying reagents mercaptoethanol, glutathione, dithiothreitol, iodoacetate and p-chloromercuribenzoate also inhibited the transport of histamine (HIS).

Additional studies revealed that the structural analogues of histamine, histidine and imidazole acetic acid (ImAA, is an intermediate of the histamine catabolic pathway), did not affect (or do it very slightly) HIS, but induced two different systems (specific for histidine or ImAA) that are also able to transport histamine.

P14-23
Pseudomonas putida OM, a new mcl-PHAs overproducer strain

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Pseudomonas putida OM was isolated from the ground surrounding an oil mill in a routine search of bacteria that accumulate mcl-poly-3-hydroxy-alkanoates (mclPHAs). When cultured in a chemically-defined medium (MM) containing glucose (60 mM) and octanoic acid (30 mM) as carbon sources, this strain was able to accumulate a huge quantity of polyoxoesters about 50% of the bacterial dry weight –bdw–. Under the same conditions, *P. putida* U, a paradigmatic strain used for decades in many genetic and biochemical studies, accumulated much less PHAs (about a 28% of bdw).

Ultrastructural studies revealed that when *P. putida* OM was cultured in different media containing ampicillin (100 mg/ml), the organism appeared as giant cells (more than 50 mm in length and 1.5 mm in width). Filamentation, which in bacteria is induced by different physicochemical or metabolic factors, is caused in *P. putida* OM by the presence of ampicillin, since in the absence of this antibiotic, the size and the length of *P. putida* OM range between the normal values (0.5–2 mm) reported for other pseudomonads. Furthermore, the acquisition of tubular morphology was not related to the synthesis of PHA since these unusual forms were also observed when this strain was cultured under non-PHA-producing conditions.

A *P. putida* OM mutant in which the fatty acid β-oxidation pathway was deleted (PpOMΔ*fadBA*) accumulated a huge quantity of PHAs (containing either aromatic or aliphatic monomers) when cultured in MM containing glucose and different n-acyl/n-aryl-alkanoic acids. Thus, the amount of poly-3-hydroxy-8-phenyloctanoate synthesized by this mutant was larger than 61% (bdw), being the most important overproducer strain that we have ever worked with. This mutant has a high biotechnological potential since it can be used for the industrial production of many unusual PHAs.

P14-24
Initial steps in deoxycholate and other bile salts catabolism in *Pseudomonas* sp. DOC21

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Pseudomonas sp. DOC21 has been isolated in our laboratory in base to their ability to use bile salts, testosterone or androstanolone as sole carbon source supporting bacterial growth. Genomic region where the steroid catabolic genes clustered has been determined. In this DNA fragment, three different genes encoding acyl-CoA ligases have been identified. We hypothesized that one or some of the acyl-CoA ligases could be involved into bile salts degradation being the enzyme(s) in charge of lateral chain activation or being involved in the last steps of steroid nucleus degradation. To get a deeper insight about these possibilities, specific deletion of these genes have been carried out using a recombinational strategy using a *sacB* containing construct previous to selection of non-lethal double recombinants in which the target gene has been deleted. Thus, mutant strains in which acyl-CoA ligase A or B are still able to grow using bile salts as the sole carbon source, and they did it at the same rate than wild type strains. However, the mutant strain in which the acyl-CoA ligase C has been deleted, although grew as well as the wild type strain when testosterone was used as the sole carbon source, was completely unable to grow when the carbon source in the media was a bile salt. These results suggest that acyl-CoA ligase C is the only AMP-forming acyl-CoA ligase involved in lateral chain degradation of bile salts, and there is not a redundant activity able to substitute it. Activation of different bile salts using a heterogously expressed enzyme has been performed.

Moreover, in base to homology at protein level, we have identified a gene encoding 3α-hydroxysteroid dehydrogenase, involved into functionalization of the 3α-hydroxy group present in the steroid nucleus of bile salts. This is a mandatory step in the initial degradation of these compounds. A deletion mutant of this gene has been tailored, being unable to grow using bile salts, but growing when testosterone or 4-androstene-3,17-dione are used as the sole carbon source. Characterization of the activity encoded in a transgene using *Escherichia coli* as host has been performed.

P14-25
A marcrolide glucosyltransferase promoting the synthesis of O-, S- and N-glucosidic compounds

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A marcrolide glucosyltransferase (*BcGT*) from *Bacillus cereus* was overexpressed and purified. The enzyme catalyzes the reaction of transferring glucosyl moiety from UDP-Glu to various acceptors to form β-configuration of O-glucosidic products. The enzyme exhibits a broad specificity on glucosyl acceptor, whereas the UDP-Glu is strictly required. Many flavonoids such as kaempferol, quercetin, apegenin, genistein, narigenin and luteolin have been tested as the glucosyl acceptor with good yield. The O-glucosylation activity is not limited to flavonoid substrates. Many phenolic compounds with different structural skeleton such as stilbenes, coumarins, steroids, biphenyl, can be glucosylated by the catalysis of *BcGT*. In addition to phenols, thiophenols and aromatic amines can also serve as glucosyl acceptor to form S- and N-glucosidic products, respectively. This enzymatic feature is particularly useful for the preparation of glucosylated compounds.

P14-26
Characterization of a novel nicotinamide mononucleotide deamidase from *Oceanobacillus iheyensis* HTE-831

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Nicotinamide mononucleotide (NMN) deamidase (EC 3.5.1.42) is one of the key enzymes of the bacterial pyridine nucleotide cycle (PNC). It catalyzes the conversion of NMN to nicotinic acid mononucleotide and ammonia, which is later converted to NAD by entering the Preiss-Handler pathway. The PNC is a network of salvage and recycling routes maintaining the homeostasis of NAD cofactor pool in the cell.

Recently, the gene encoding for the enzyme responsible of nicotinamide mononucleotide activity (PncC) was elucidated. Despite the broad distribution of predicted functional PncC in bacterial kingdom, NMN deamidase activity has only been described and characterized in a few gamma-proteobacteria.

This work describes the cloning, overexpression, purification (120 mg purified protein/L of cell culture) as well as the molecular characterization of the novel NMN deamidase from the extremophile *Oceanobacillus iheyensis* (OiPncC), using a novel spectrophotometric method, in comparison with the tedious HPLC-based method.

OiPncC showed a K_m value for NMN of 0.16 mM and a k_{cat}/K_m value of 1.1/mM/sec. The enzyme was active over a broad pH range, especially at basic pH′s, being its optimum pH 7.4 but maintaining 90% of activity at pH 10.0. Incubation of OiPncC at different pH′s revealed that the enzyme was surprisingly stable at basic pH′s, maintaining around 50% of residual activity after 72 hours at pH 8–10.6. Curiously, OiPncC activity could still be measured after 3 weeks at pH 10.0. OiPncC shows good activity from 37 to 60°C, falling quickly above 65°C but maintaining 60% of residual activity after 24 hour incubation at 45°C.

Little is known about the molecular structure of NMN deamidases. A comparative analysis of its sequence and its homology modelling of OiPncC with other predicted bacterial NMN deamidases found in the databases was carried out in order to shed light on the classification of those enzymes.

This study was partially supported by MINECO-FEDER (BIO2010-22225-C02-01) and Programa de Ayuda a Grupos de Excelencia de la Región de Murcia, of Fundación Séneca (04541/GERM/06, Plan Regional de Ciencia y Tecnología 2007-2010). G.S.C is a holder of a predoctoral research grant (FPU) from Ministerio de Educación, Cultura y Deporte, Spain. M. I. G. G. is holder of a predoctoral research grant associated to the above project from Fundación Séneca.

P14r-27
Dihydroquercetin-modified polyethylenimine as material for tissue engineering

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Polyethylenimine (PEI) is positively charged polymer, which is used in genetic engineering for DNA and RNA delivery and in tissue engineering for stimulation of cell adhesion [1, 2, 3], but it shows rather high level of toxicity inducing mitochondrial membranes destabilization [3]. The aim of this work was to prepare PEI-based film supporting cell growth. Polyphenolic compound dihydroquercetin (DHQ) was offered as an agent which cross-links PEI chains and decreases its toxicity. Final PEI concentration was 6.6% and DHQ was 0.26–3.3% (molar ratios PEI:DHQ were 2.4:100–30.3:100). Prepared films washed in water or PBS to remove unbounded oxidized DHQ and in protein (BSA) solution for increasing supports biocompatibility. Biocompatibility of the films was tested using HaCaT cell line. Films containing 1.1–0.55% DHQ showed good transparency and insolubility in PBS, water, cultural medium and ethanol. This film weakly detached from glass surface when aqueous solutions were added. Two and 3-fold decreasing PEI and DHQ concentrations resulted to preparing thin and more transparent but stable films showing more affinity to glass. We hope oxy and hydroxy groups of DHQ form strong hydrogen and covalent bonds with PEI amino groups. Cells grow on the surface of the abovementioned films including films with entrapped BSA while unmodified PEI was toxic and resolved in medium for cell cultivation. PEI-DHQ films can be applied for modification of non-adhesive surfaces of the implants for tissue engineering.

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P14-28
Engineered *Geobacter sulfurreducens* strains towards the improvement of metal bioremediation pathways

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Geobacter species have become a potential target for bioremediation and biotechnological applications towards the decontamination of different environments due to its impressive respiratory versatility [1]. This includes the unusual ability to oxidize organic compounds using Fe(III) or other metal oxides as terminal electron acceptors. More than one hundred putative *c*-type cytochromes were identified in the genome of *Geobacter sulfurreducens* (*Gs*) and a family of five periplasmic triheme cytochromes (designated PpcA-E) was showed to play a crucial role in *Gs* respiration skills by driving electron transfer from the cytoplasm to the cell exterior and assisting the reduction of extracellular acceptors [2,3].

The functional properties of PpcA have been already characterized and this protein was shown to have the thermodynamic properties necessary to perform e^-/H^+ energy transduction in addition to electron transfer [4]. A large family of PpcA mutants was also characterized and those showing significant enhancement in their e^-/H^+ energy transduction profiles or in the order of oxidation of the redox centers were selected for *in vivo* studies. Plasmid pRG5 [5], was used for expression *in trans* of the PpcA mutants in the *Gs* strain DL3 with *ppcA* gene knocked-out [6]. In this communication the data gathered so far on the phenotypic analysis of the different engineered *Gs* strains will be presented. This work will contribute to the understanding of *Gs* respiratory chains and to design more efficient proteins for improved *Gs* metal bioremediation pathways.

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P14-29
Biotechnological tools from viral factories

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Most viruses build their own intracellular compartments where viral components are concentrated to assemble the viral progeny more efficiently. These structures, termed viral factories or

viroplasms, are easily seen under the microscope as dense cytoplasmic inclusions, and are devoid of membranes or cellular organelles.

The nonstructural protein muNS forms the scaffold for avian reovirus viroplasms and is able to form intracellular inclusions without any help from other viral or cellular proteins [1,2]. Our extensive characterization of the domain composition of muNS [2] allowed us to develop a molecular tagging system (IC-tagging) for targeting proteins to cytoplasmic muNS-derived inclusions. Combination of the IC-tagging and inclusion-targeting system with our previously developed method to purify muNS-derived inclusions [2], produced a simple, versatile and inexpensive method for purifying proteins. The purified polypeptides can be obtained either soluble or as particulate material (inclusion-integrated). Such purification system can be particularly useful for purifying immobilized enzymes, therapeutic proteins or to produce particulate material 'decorated' with different epitopes for immunization purposes.

Aditionally, we have adapted our IC-tagging system for detecting protein-protein interactions in the cytoplasm of living eukaryotic cells [3]. The relocation caused by inclusion integration raises the idea of using IC-tagging for *in vivo* functional studies by creating intracellular compartments for protein-ligand sequestration. We have engineered the muNS-derived proteins to form inclusions also inside the cell nucleus, to be used for detecting protein-protein interactions, or to form the mentioned compartments, in the natural environment of nuclear proteins.

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P14-30
Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases

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Biophysical and mechanistic investigation of RNA function requires site-specific incorporation of spectroscopic and chemical probes. In nature, a particular class of RNA methyltransferases, box C/D ribonucleoprotein complexes (C/D RNPs), direct AdoMet-dependent site-specific 2′-O-methylation to numerous biological sites (rev. in 1). Precise baise pairing of a guide RNA and the substrate selects the target nucleotide. We have combined the specificity of C/D RNP machinery with synthetic AdoMet analogs to incorporate a reactive group which could be further appended with a desired label. Therefore we have *in vitro* reconstituted a C/D RNP from the thermophilic archaeon *Pyrococcus abyssi* and demonstrated its ability to transfer a prop-2-ynyl group from a synthetic cofactor analog (2) to both the wild-type and newly programmed target sites in model tRNA and pre-mRNA molecules. Target selection of the RNP was programmed by changing a 12 nt guide sequence in a 64-nt C/D guide RNA leading to efficient derivatization of three out of four new targets in each RNA substrate. We also show that the transferred terminal alkyne can be further appended with a fluorophore using a bioorthogonal azide-alkyne 1,3-cycloaddition (click) reaction. The described approach (3) for the first time permits synthetically tunable sequence-specific labeling of RNA with single-nucleotide precision.

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P14-31
Development of a highly specific DNA nicking nuclease

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Gene correction by double strand break- or, preferably, single strand nick-induced homology-directed repair requires highly specific nucleases (e.g. engineered homing endonucleases, zinc-finger nucleases or TALE nucleases). Although engineering of such highly specific nucleases has been successful, the probability of non specific DNA cleavage (off-target cleavage) still precludes gene therapy of monogenetic diseases of humans. In an effort to develop novel highly specific nucleases we fuse DNA binding modules with extended recognition sequences, and DNA cleavage modules, e.g. endonucleases with appropriate cleavage characteristics, to generate efficient tools for genome engineering. One example that illustrates our approach is the fusion construct of an inactive variant of the homing endonuclease I-SceI (I-SceI*) as a DNA binding module and MutH as a cleavage module. I-SceI recognizes an 18 bp sequence and is specific enough to address a unique site in the human genome. MutH is a DNA mismatch repair enzyme and is responsible for nicking the DNA at a GATC site in the vicinity to a base pair mismatch. Since MutH needs the guidance of the MutS/MutL mismatch recognition cascade to become catalytically active, the fusion enzyme MutH-I-SceI* should not be able to cleave unaddressed GATC sites under physiological conditions. Only if a GATC-site is addressed by an I-SceI recognition site, the fusion enzyme should be targeted to the addressed site by the binding module I-SceI*, allowing MutH to catalyze a site specific nick. Our engineered MutH-I-SceI* constructs were tested for activity and specificity *in vitro*. It could be shown that the fusion construct has a clear preference for nicking the addressed target site, whereas unaddressed GATC sites remain uncleaved.

P14-32
Obtaining an analogue of the UBP4 protease gene using synthetic fragments of DNA created by DnaSynth software employing evolutionary algorithms

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The objective was to obtain a shorter gene of the deubiquitinating UBP4 protease analogue. This gene sequence was described in 1995 (GeneBank: CAA89098). A synthetic gene is a DNA strand with a specific sequence. It is synthesized *in vitro*, without requiring an initial DNA template. Redesign of de novo synthesized DNA molecules is more cost efficient and flexible than production of molecules using traditional methods.

The activity of UBP4 proteases is very specific. The UBP4p protease is an enzyme, which cleaves ubiquitin from proteins fused to its C-end. The whole gene is 2778 bp long, but a shorter analogue that is only 276 bp long was designed.

DnaSynth software was used to synthesize the analogue of *Saccharomyces cerevisiae* yeast Ubp4 protease gene. The paper presents DnaSynth software, which can design an optimal DNA sequence and find the long DNA assembly protocol. A codon change may cause a change in the optimal assembly protocol.

The software was tested *in vitro*, on the generated data. Eleven fragments were designed by the software and the total length of fragments was 276 nucleotides. These fragments could be mixed in one tube without the risk of incorrect binding thus permitting one step synthesis.

The obtained DNA sequence of the Ubp4 analogue gene was confirmed by means of sequencing.

P14-33
Development of a new affinity immobilization procedure on functionalized graphite electrodes for proteins tagged to choline-binding modules

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The development of new specific immobilization techniques of proteins on electrodes is an important task in the fabrication of efficient amperometric biosensors. In this sense, the functionalization of solid surfaces combined with the use of affinity tags is a promising alternative. Here we evaluated the binding and stability of β-Galactosidase (β-Gal) tagged to the choline-binding domain C-LytA (Clyt-βGal) to choline functionalized monolayers assembled over graphite electrodes. Cyclic voltammetry using 4-aminophenyl β-D-galactopyranoside (PAPG) as substrate indicated that the fusion protein Clyt-βGal was readily immobilized and remained functional. The binding turned out to be specific, as the protein could be selectively eluted in the presence of competitor choline, but not in equivalent concentrations of NaCl. Upon elution, the treated electrode was ready to immobilize a new fresh batch of protein. Moreover, non-fused β–galactosidase only bound to the electrode in residual amounts. The catalytic activity of the fusion protein adsorbed on the functionalized graphite electrodes was evaluated at different concentrations of PAPG by cronoamperometry and over the time. The general response of the immobilized enzyme showed a sigmoidal behavior, with a linear response at low concentrations of PAPG (0.1–1.0 mM) and a saturation of the system at higher substrate concentrations. These results constitute a proof-of-concept that shows the feasibility of the C-LytA system to immobilize enzymes on functionalized graphite surfaces in order to construct enzymatic electrochemical cells and biosensors that can be easily regenerated and reused after the eventual protein inactivation simply by washing with choline and reloading with a fresh preparation.

P14-34
Expression of neuraminidase of influenza virus in *Escherichia coli* cells by removing of transmembrane domain of the protein

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Neuraminidase (NA) is next to hemagglutinin (HA) the main surface glycoprotein of influenza A virus. NA promotes influenza virus release from infected cells by removing sialic acids from the host cell surface. Expression systems based on prokaryotic cells are not widely used to obtain this protein. Heterologous expression of NA has been performed mainly in insect or mammalian cells. Our aim was to obtain expression of neuraminidase in *Escherichia coli* cells. To achieve this goal we created several expression vectors based on truncated gene of nauraminidase.

According to the sequence of neuraminidase of Influenza A virus (Q6J8D5) from UniProtKB the transmebrane region is located from 7aa to 27aa. Analysis of the sequence in SOSUI server indicated transmembrane regions from 11aa to 33aa. Analysis of the sequence in TopPred0.01 program indicated two certain fransmembrane region from 7aa to 27aa and from 59aa to 79aa. We constructed NA expression vectors based on pIGDMCT7RS (DQ485721 GeneBank). To obtain the expression of neuraminidase in *Escherichia coli* cells we truncated of 33 amino acids and 80 amino acids from C-terminus of NA protein. We constructed expression vector with full length protein as well. We obtained the expression of both truncated proteins but not full neuraminidase. Protein was produced in *Escherichia coli* as inclusion bodies.

P14-35
Production and evaluation of polyclonal antibodies against alpha toxin oligomer from *Clostridium septicum*

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Clostridium septicum is an anaerobic Gram-positive bacterium, pathogenic to humans and animals. The protein alpha-toxin is the main virulent factor of this bacterium having hemolytic, lethal and necrotizing activities, being used as an antigen to develop animal vaccines.

Most of the antibodies commercially available today against this toxin do not offer the specificity required and, for this reason, it is of interest to produce and purify new antibodies against clostridium septicum alpha-toxin.

Since toxin is known to aggregate *in vivo* forming active oligomers, the aim of this study was to design a polyclonal antibody able to specifically detect the alpha toxin oligomer.

The presence of alpha-toxin oligomer was detected in cultures by SDS-PAGE, identified by Mass Spectrometric methods and purified oligomer was used to produce polyclonal antibodies in rabbits. Specificity of the polyclonal antibodies obtained was tested for western and slot-blot techniques.

Quantification of alpha-toxin revealed that it could de detected at concentrations as low as 16 ng/ml and the reaction was highly specific.

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P14r-36
Improvement of thermal stability of *Kluyveromyces lactis* β-galactosidase

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β-D-Galactosidase (E.C. 3.2.1.23) from the yeast *Kluyveromyces lactis* is one of the most used enzyme in food industry because of its ability to hydrolyze lactose in milk or derived products like cheese whey. It has been widely used to obtain lactose free-milk and dairy products. β-galactosidases have also trangalactosylation activities that make them very attractive for the obtaining of probiotics milk derivates like galactooligosaccharides (GOS). The industrial production of GOS needs high concentrations of lactose and high temperatures because of the low solubility of this disaccharide. However, the low stability of β-D-Galactosidase of *K. lactis* limits the use of the enzyme in applications that need high temperatures, like production of GOS. In order to improve the thermal stability of the protein, we have studied its recently resolved structure (J. Struct. Biol., 177: 392–401, 2012) and applied the B-FIT (B-Factor Iterative Test) (Angew. Chem. Int. Ed. Engl., 45: 7745–7751, 2006) approach for target selection and mutagenesis based on the crystallographic B-Factor information and other directed evolution methods. These approaches have allowed us to identify some variants that show a slightly increase in melting temperature. This improvement in thermal stability constitutes a first step in the engineering of the protein towards a better industrial enzyme.

P14-37
Production and characterization of a novel pullulanase from *Hypocrea jecorina* QM9414

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Starch-hydrolysing enzymes such as pullulanases, amylases, and glucoamylases play an important role in food, chemical and pharmaceutical industries. Pullulanases (pullulan-6-glucanohydrolase [EC 3.2.1.41]) are classified as type I or type II depending on their abilities to degrade α-1,4 or α-1,6 glycosidic linkages in starch, amylopectin, and related oligosaccharides. The filamentous fungus *Hypocrea jecorina* (anamorph *Trichoderma reesei*) is a potent producer of cellulolytic and hemicellulolytic enzymes (1-3). In this study, pullulanase induction was studied for the first time at *H. jecorina* QM9414. Optimization of pullulanase production conditions from *H. jecorina* was carried out in shaking cultures containing amylopectin, starch or pullulan; the optimum concentration value was found to be 0.5% (w/v) amylopectin, pH 6.5, temperature 30°C. Pullulanase was purified 11 fold from this microorganism. Some properties of the purified enzyme were investigated. The purified enzyme showed a single band on SDS-PAGE with an estimated molecular mass of about 130.56 kDa. The optimum pH for the purified enzyme was 6.5. The optimum temperature was between 35 and 65°C. Michaelis-Menten constant and maximum velocity values were 10.7 mg/ml and 3.3145 ΔA/min, respectively, with amylopectin as substrate. Heat inactivation studies showed that *H. jecorina* pullulanase was stable at 40–70°C for 30 min. However, the enzyme lost about 33% of its activity at 80°C and about 43% of activity at 90 and 100°C, for the same incubation period. Pullulanase activity was stimulated by CoCl2, NiCl2, KI, NaCl, MgCl2, and LiSO4. The enzyme was slightly inhibited by urea, CaCl2 and 2-mercaptoethanol.

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P14-38
Development of enzyme replacement therapy for metabolic disease propionic acidemia

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Propionic Acidemia (PA) is a hereditary metabolic condition that results from the deficiency of Propionyl CoA Carboxylase (PCC), the mitochondrial enzyme responsible for degrading the metabolic intermediate Propionyl CoA derived from multiple metabolic pathways. The consequent accumulation of this toxic metabolite results in severe symptoms such as impairment to mental development as well as severe ketoacidotic attacks. Currently, except for drastic surgical and dietary intervention that can only provide partial relieve to PA, no other form of therapeutic option is available for this genetic disorder. Similar to other classical mitochondrial disorders, the specific localization of PCC within the mitochondria poses as a major hurdle to the delivery of potential enzyme replacement therapeutics. We proposed to examine a novel approach in protein delivery by utilizing cell penetrating peptides and mitochondrial targeting sequences to design and produce specific fusion PCC subunits, capable of translocating and localizing across membranes so as to develop the first enzyme replacement therapeutic for PA. More importantly, PA will serve as a model disease for understanding and development of subsequent therapeutic strategies for other mitochondrial disorders, all of which are currently incurable.

P14-39
Exploring the cellulase diversity in *Penicillium* sp. for 2nd generation bioethanol production

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Cellulose is the most abundant organic compound on earth, contained in leaves, stems, and stalks of plants and trees. Since it is not used for food purposes, cellulosic ethanol is expected to be less expensive and more energy-efficient than today's ethanol because it can be made from low-cost feedstocks. Chemically, cellulose is a homopolysaccharide of β-1,4-linked D-glucose

residues, whose full enzymatic hydrolysis requires three major types of enzymatic activity: endoglucanases, exoglucanases and β-glucosidases.

The synergistic action of these enzymes is essential for the generation of monomeric sugars from cellulose, which can be converted into ethanol by microbial fermentation. Although there are different commercial cellulases it is necessary to increase their efficiency for lignocellulosic biomass treatments. For this reason, research on cellulases characterization and large-scale production has been considered a pivotal step on 2nd generation bioethanol production. Nowadays, although all components of the extracellular cellulose complex are essential for cellulose hydrolysis, β-glucosidase is either lacking or present in relatively small amounts in the commercial available cocktails. The new discovered enzymes can be used as a new formulation or either complement existing enzyme mixtures.

After a large fungal screening, a *Penicillium* strain has been selected for its ability to produce high levels of cellulases. Two endoglucanases and two β-glucosidases have been purified and biochemically characterized. One of the β-glucosidase is able to degrade xylan derived subunits as well as typical substrates reported for these enzymes.

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P14-40
Antifungal activity of the plant specific insert domain against phytopathogenic fungi

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The plant specific insert (PSI) is a region of approximately 100 amino acids residues present in typical plant aspartic protease (AP) precursors. It has been anticipated that this domain may function as a defensive 'weapon' against pathogens. Indeed, the capacity of cardosin A PSI domain to interact with phospholipid membranes and to induce permeabilization has been previously demonstrated in our lab. Moreover, it was also reported that the PSI domain of potato AP exerts antimicrobial and antifungal activity on both plant and human pathogens.

Given the economic impact of the deleterious effect of plant pathogens in crop cultures, the identification of new compounds with antifungal activity has a high biotechnological value. Therefore, the goal of this work is to evaluate the antifungal activity of the PSI domain of cirsin (AP from *C. vulgare*) against different phytopathogenic fungi isolated from vineyards.

The cDNA encoding cirsin PSI domain fused to a C-terminal His-tag was cloned into pKLAC1 vector for production in *K. lactis*. The signal peptide of the α-mating factor was used to direct recombinant protein to secretion. *K. lactis* positive transformants were selected by PCR and expression levels were screened by WB. Transformants displaying higher expression levels of PSI were selected. Evaluation of the antifungal activity of PSI was initially assessed by co-growth assays of the selected *K. lactis* strains with each fungus. Antifungal activity was observed towards the fungi *L. Infectoria*, *A. alternata*, *D. biseptata* and *P. herbarum*. This bioactivity is being currently characterized by disk diffusion and agar dilution susceptibility tests using purified recombinant PSI. The potential of cirsin PSI domain as a new fungicide for crop disease control will be discussed.

P14-41
Chemo-enzymatic approaches to genome-wide profiling of cytosine modifications

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Methylation of cytosine residues in CG sequences is part of an epigenetic regulatory mechanism in high eukaryotes. Aberrant DNA methylation correlates with a number of pediatric syndromes and cancer, or predisposes individuals to various other human diseases. Recent studies showed that CG sequences can contain 5-hydroxymethylcytosine, which might represent another level of epigenetic regulation. However, research into the epigenetic misregulation and its diagnostics is hampered by the lack of adequate analytical techniques that are able to unequivocally distinguish and map both cytosine modifications in DNA. We have developed a new strategy for parallel genome-wide analysis of cytosine modifications in genomic DNA based on covalent capture of unmodified or hydroxymethylated CpG sites. The covalent tagging of unmodified CpG sites with biotin was performed using an engineered version of a CpG-specific DNA cytosine-5 methyltransferase and a chemically synthesized analog of the *S*-adenosylmethionine cofactor. Analysis of the enriched human unmethylome on tiling DNA microarrays showed that the new approach shows important advantages over existing methylation profiling techniques. For analysis of 5-hydroxymethylcytosine, we have redesigned the CpG-specific DNA cytosine-5 methyltransferase reaction for the exchange and labeling of 5-hydroxymethyl groups on cytosine residues in DNA using non-cofactor like exogenous compounds [1,2]. These novel approaches open new ways for genome-wide analysis of cytosine modifications using modern sequence analysis techniques.

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P14-42
Targeting telomerase reverse transcriptase: a molecular approach for gene therapy in HCC

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Hepatocellular carcinoma (HCC) is currently one of the most common worldwide causes of cancer death counting 560,000 new cases per year. Orthotopic Liver Transplantation (OLT) or liver resection represent the best treatments for HCC. However, most patients cannot undergo surgical treatments and chemotherapy represents the alternative. Unfortunately the establishment of multidrug resistance leads to new therapeutically approaches such as gene therapy.

Targeting essential and specific cancer-related genes represent the new challenge in anticancer molecular techniques. Based on literature we selected four candidates for gene therapy such as

aurora kinase A, survivin, midkine and telomerase. By screening the mRNA expression of these genes in of a cohort of 21 HCC patients we then selected telomerase as the best candidate for the gene silencing therapy since its expression decreased significantly from tumoral to distal tissue ($p < 0.01$ and $p < 0.001$ respectively), and was not expressed in normal tissues. Telomerase is a reverse transcriptase responsible of telomere maintenance which, in contrast to its physiological expression pattern, becomes upregulated in many types of cancer.

The telomerase catalytic subunit (hTERT) was silenced in poorly differentiated HCC derived cells (JHH6) using 25, 50 and 100 nM of a non-inflammatory siRNA. A 72h silencing significantly reduced hTERT mRNA expression ($p < 0.001$) and enzyme activity ($p < 0.01$). The treatment induced morphological changes: undifferentiated cells (fibroblast-like) became slightly more differentiated (hepatocyte-like), furthermore albumin (an hepatic hallmark) became detectable.

Our designed siRNA resulted more effective in killing cancer cells than doxorubicin, the widely used antineoplastic drug ($p < 0.01$). Among the 63% surviving cells there is an increased percentage of cell cycle arrested cells ($p < 0.01$). All data were validated using telomerase negative cells.

This work evidences the pivotal role of hTERT not only in maintaining telomere length but also in cell survival and proliferation.

P14-43
Production of recombinant human growth hormone fusion protein in *Escherichia coli* in a laboratory scale fermenter

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Human growth hormone (hGH) was one of the first recombinant proteins approved for the treatment of human growth disorders. Its size of only 191 amino acids, presence of only two disulphide bonds and absence of posttranslational modifications make *Escherichia coli* a host of choice for both small and large scale production. By the application of rational design, an effective T7 expression system utilizing arabinose as an inducer had been developed. After construction of the *E. coli* expression system, its optimization and product confirmation, the first steps toward process scale up had been taken. Using a laboratory fermenter with a working volume of 2 liters, parameters such as cultivation media composition, pH, oxygen saturation, temperature, concentration of glucose and arabinose, suitable induction OD_{600} and the overall duration of cultivation have been assayed and optimized to certain degree. The result was the high level expression of recombinant human growth hormone fusion protein, in the form of inclusion bodies as well as a soluble protein. Presented work describes optimal expression conditions and compares these two approaches – production of soluble or insoluble protein – in terms of subsequent downstream processing, isolation and purification of the product. This work is the result of the project implementation: ''Production of biologically active agents based on recombinant proteins'' (ITMS 26240220048) supported by the Research and Development Operational Program funded by the ERDF.

P14-44
Expression, purification and characterization of a sirtuin from *Oceanobacillus iheyensis* HTE-831

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Sirtuins are protein deacetylases, which are dependent on nicotine adenine dinucleotide. They are phylogenetically conserved from bacteria to humans. These enzymes catalyze a unique reaction in which NAD^+ and acetylated substrate are converted into deacetylated product, nicotinamide and a metabolite *O*-acetyl ADP-ribose. Sirtuin of *Oceanobacillus iheyensis* forms insoluble inclusion bodies when expressed in *E. coli*, resulting in low yields of soluble protein. In this study we have developed several sirtuin expression systems that generate a high amount of soluble protein and use a simple purification scheme. We cloned this sirtuin fused to either maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin protein (Trx), not fused to either proteins in pET24b and C-Terminal His-tag. Furthermore, we expressed in several expression *E. coli* strains. Under optimized conditions, the yield of soluble MBP-Sirt and Trx-Sirt were the highest. These results demonstrate that the use of MBP/Trx-fusion proteins provide an approach to generating high yields of soluble and functional of this sirtuin. An efficient purification method was also developed, with a good yield. The molecular mass of purified OiSirt, after the MBP-tag removal with 3C protease, was determined by SDS-PAGE (24 kDa) and by gel filtration (~23.5 kDa) and by HPLC/ESI/ion trap (24.4 kDa), confirming the monomeric nature of OiSirt. The enzyme was active towards synthetic acetylated peptides in the presence of NAD^+, rendering OAADPr and nicotinamide as a products. Thermal stability was also carried out by a thermal melt assay using Sy-Pro Orange.

A comparative analysis at the level of sequence and homology modelling of OiSirt and other predicted close-related sirtuins found in the databases was carried out.

This study was partially supported by MEC (BIO2010-22225-C02-01) and Programa de Ayuda a Grupos de Excelencia de la Región de Murcia, de la Fundación Séneca (04541/GERM/06, Plan Regional de Ciencia y Tecnología 2007-2010). G.S.C is a holder of a predoctoral research grant (FPU) from Fundación Séneca, Murcia, Spain. M.I.G.-G is a holders of predoctoral research grants associated with the above project from Fundación Séneca.

P14-45
The effects of EGCG and DHA in mitochondrial function in L6 skeletal muscle cells

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It is well known the beneficial effects of the natural antioxidant epigalocatequin-gallate (EGCG) in the metabolic syndrome reversing the disease, and also those of the polyunsaturated fatty acid docosahexanoic (DHA), which improve biochemical markers of metabolic syndrome. To investigate these effects separately and in combination throw the muscle, a tissue which plays an important role in the energy metabolism and fatty acid metabolism; we decide to study the effect of EGCG and DHA focusing in mitochondria function in the L6 skeletal muscle cells. So, we

added an optimal concentration of 25 μM of ECGC and 25 μM of DHA, alone or in combination in the L6 cell culture during 4 hours. After the treatment, cells were analyzed in different ways, in aim to see the functionality of mitochondria system. The oxygen consumption was measured *in vivo*, with intact cells, by a high resolution respirometry; different states were recorded in intention to see how DHA and EGCG interact with the electron transport chain. Also, the mitochondrial gene expression was analyzed by quantitative mRNA expression, conducted via TaqMan RT-PCR. Results, conclude that in the ROUTINE respiration, supported by the substrates of the culture media, the oxygen consumption was significantly decreased in DHA and DHA + EGCG groups in comparison with control and GSPE groups. In the LEAK state, when oligomycine was added, in aim to inhibit mitochondrial ATP production, the oxygen consumption was also decreased in all groups compared to ROUTINE state, and again the oxygen consumption was significantly lower in DHA and DHA + GSPE groups than in control and GSPE groups. There were no significant differences in ETS state, when FCCP is added to uncouple the oxidative phosphorylation. Taken all these results together, cells seem to be affected when were treated with DHA, decreasing the oxidative phosphorylation. By the other hand, EGCG did not alter the functionality of mitochondrial respiration. Besides this, mRNA expression of UCP3 and UCP2 were significantly overexpressed in DHA group, compared with control group, and also in DHA + GSPE group in the case of UCP3 mRNA expression. The less oxygen consumption in DHA group and DHA + GSPE group could be correlated with the UCPs overexpression. These effects agree with the uncoupling of ETC with ATP synthesis due to the DHA treatment. Furthermore, a slight but statistically significant repression of ANT2 gene expression was observed in the two groups in which DHA was administered, suggesting a low interchange between ATP and ADP through mitochondrial membrane. This statement is reinforced in the DHA + GSPE group, where the ATPase expression was also repressed, which agrees with a lower ATP synthesis.

P14-46
A novel viral vector for the superproduction of proteins of interest in plants

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Different fields of modern biotechnology (vaccine, drug, nanomaterials manufacturing) require effective approaches for protein production. Viral vector provided proteins of interest expression in plants offers several advantages over the rest of the approaches: lack of animal pathogens contamination hazard and relatively low-cost manufacture. *Alternanthera mosaic virus* (genus *Potexvirus*) is a flexuous, rod- shaped virus with a positive sense RNA genome. The new strain of *Alternanthera mosaic virus* – AltMV-MU (GenBank FJ822136.1) was previously described in our lab. We found that the virion-derived AltMV-MU coat protein (cp) was readily assembled into RNA-free virus like particles (VLPs). The new AltMV-MU based vector (AltMV-vector) with «deconstructed» type of architecture (lacking triple gene block) was constructed in the current work. To obtain a higher expression level, the gene of interest was placed under control of multiple subgenomic promoters (sgp). To our knowledge, this is the first example of multiple sgp control of a target gene expression in a plant viral vector. The AltMV-MU cp was superexpressed by AltMV-vector in agroinjected *Nicotiana benthamiana* leaves. The amount of AltMV cp was sufficient for efficient VLP formation. Apparently, AltMV-vector could be utilized for superproduction of VLP's, which, in particular, are used in nanobiotechnology for nanovaccines and nanomaterials development. A foreign protein – the receptor binding domain of the hemagglutinin of the influenza virus A/California/07/2009(H1N1) strain, was superexpressed by the AltMV-vector in plant leaves. In summary, our data indicated that the technology of multiple subgenomic promoter control was efficient for superexpression of genes of interest by plant viral vector.

P14-47
The effect of the Gcd, *zwf-1* and *zwf-2* genes on alginate biosynthesis and growth of *Pseudomonas fluorescens*

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Pseudomonas fluorescens has the ability to produce the linear polymer alginate, although the genes are normally not expressed. By mutating a wild-type strain of *P. fluorescens* we have been able to obtain the strain Pf201 with good constitutive alginate production level. Alginate is synthesized from fructose-6-phosphate, and thus competes with the central carbon metabolism for fructose-1,6-diphosphate. This motivated our present study on how the flow through the various early pathways of the carbon metabolism would influence alginate yield. This was performed by analyzing how the contribution of glucose dehydrogenase (Gcd) and the two glucose-6-phosphate dehydrogenases (G6PDH) (Zwf-1/-2) affect growth and alginate production, by studying mutants where one or more of these genes were deleted. Alginate production and growth data on three different carbon sources, glucose, fructose and glycerol, were obtained for the studied strains. The results showed that the *gcd*-mutant both grew better and produced much more alginate than Pf201 on glucose. A possible explanation is that the periplasmic Gcd enzyme converts glucose to gluconate leading to an acidification of the medium, which negatively affects growth. Our data also show that Zwf-1 contributes to most of the G6PDH-activity, since deleting *zwf-2* alone did not have any significant effect on growth or alginate production in any medium, while deletion of *zwf-1* had a significant impact on growth and alginate production on all three tested carbon sources.

P14-48
Set of high-throughput T-vectors for production of recombinant proteins in plants

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Modern biotechnology is interested in production of high quality recombinant proteins in sufficient amounts. Usage of plants as biofactories is one of the perspective approaches to obtained it. We have created an original set of vectors for transient expression of recombinant genes in *Nicotiana benthamiana* leaves. It considerably simplifies cloning of genes of interest to a one-step method allowing direct ligation of PCR products into expression plasmid vectors with required combinations of features. Each vector carries one of the tags for purification of recombinant protein by affinity chromatography (HisTag, HA, FLAG, Myc) and localization of recombinant protein in different cellular compartments (cytoplasm, apoplast, ER). Moreover, one vector could be

used for co-expression of multiple proteins in three different compartments: cytoplasm, ER and nuclear, through the highly effective tool – 2A oligopeptide which forms a self-processing polyprotein. Our vectors are based on a vector containing 35S promoter located in T-DNA and tobacco mosaic virus RNA. It can be efficiently delivered to the plant cells by agroinfection of *Agrobacterium tumefaciens* and is capable to produce high levels of recombinant proteins in plants. Furthermore, vectors possess the negative selection marker, gene *ccdB*, which encodes a potent cell-killing protein to eliminate the possibility of self-ligations. It significantly increases the number of clones carrying an insert and makes it possible to perform a high-efficiency cloning. Thus, the set of vectors could become a powerful tool for high-throughput transient expression of recombinant proteins in plants in biotechnology and functional genomics studies.This work was supported by grants from Presidium of RAS ('Nature') and RFBR (#10-04-01195-a).

P14-49
The purification of biological active recombinant human endostatin from *E. coli*

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Endostatin, 20 kDa fragment carboxyl terminus derived from collagen XVIII, is an angiogenesis and tumorigenesis inhibitor that can restrict VEGF-induced proliferation and migration by inactivation of the endothelial nitric oxide synthase and regress a tumor growth. Zinc ion at N-terminus of endostatin play a critical role on the activity of angiogenesis and tumor repression in vascular endothelial cells. Therefore, endostatin has been purified to suppress the tumor-induced angiogenesis as therapeutic protein. The purpose of this study is to purify and identify the biological active rh-Endostatin. We for the first time purified rh-Endostatin as soluble form with human protein disulfide isomerase (hPDI) and characterized rh-Endostatin by MALDI-TOF MS analysis. Finally, we hope the purification of biological active rh-Endostatin as soluble form will provide opportunities for tumor-induced angiogenesis treatment.

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P14-50
Engineering outer membrane vesicles

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Outer Membrane Vesicles (OMVs) are closed spheroid particles of a heterogeneous size (10–300 nm in diameter) that arise through bulging and pinching off of the outer membrane of Gram negative bacteria. They are composed of outer membrane and periplasmic proteins and have proposed roles in virulence, inflammation and envelope stress response. It has been shown that purified OMVs elicit protective immune response against several pathogens thanks to their immunogenic properties, self-adjuvanticity and ability to be taken up by mammalian cells, rendering them attractive candidates for vaccine delivery platforms. We have selected a number of proteins from different bacteria, both Gram positive and Gram negative, belonging to different cellular compartments as model systems to demonstrate the possibility to deliver them to the OMV in native conformation. To this aim the genes coding for these proteins have been manipulated to express them fused to a heterologous leader sequence for secretion. When cloned in *Escherichia coli* BL21 strain using pET vector all proteins were found in the OMVs. Here we show that the OMV-associated recombinant proteins preserved their biological activity and immunological properties as judged by different immune and enzymatic assays.

P14r-51
Unravelling the metabolic differences between *Escherichia coli* K12 and BL21

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Escherichia coli BL21 strain is a recognized work horse in Biotechnology due to its ability to yield high recombinant protein titers. Several groups have aimed at identifying the metabolic features responsible for this high productivity. Comparison with the prototype *E. coli* K12 strain has revealed a much lower acetate overflow in BL21. In the last years, acetylation of proteins has emerged as a novel mechanism for metabolic regulation. The enzyme responsible for acetate overflow, acetyl CoA synthethase (Acs), as well as many other central metabolism enzymes are regulated by this system. We have recently demonstrated that the protein acetylation system and central metabolism are transcriptionally linked. The aim of this work was unraveling the role of protein acetylation in determining the metabolisms of *E. coli* BL21 and K12. The expression of the protein deacetylase *cobB* gene in the BL21 strain is higher than in K12, especially in stationary phase, while the expression of the protein acetylase *patZ* gene was slightly lower. Genomic analysis showed the insertion of a transposon within the *nagK-cobB* transcriptional unit in BL21. Single and double gene knock-out mutants further demonstrated the differences between the two genetic backgrounds. For instance, deletion of *cobB* in BL21 transformed it into a high acetate producer. Deletion of *patZ* in BL21 accelerated growth, which suggests that this enzyme controls the activity of several metabolic pathways. The wild type and deletion strains were characterized using gene expression, promoter activity assays, enzyme activity and metabolite profiles. In addition, the production of eGFP in the strains was also determined, demonstrating the global relevance of metabolic regulation by protein acetylation in BL21. Altogether, this work contributes to the understanding of the reasons underlying the differences between *E. coli* K and B strains. In addition, we present the first evidence that regulation of bacterial metabolism by protein acetylation is crucial for determining the performance of bioprocesses, opening new opportunities in Biotechnology.

P14-52
Saccharomyces cerevisiae – a perfect host for expression and simple one-step purification of active tag-free human BiP/GRP78 protein

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Growing demand for various recombinant proteins and their qualities necessitates search for the novel and/or better expression systems even for the proteins which have well established production protocols. Native human BiP/GRP78 protein for various purposes is often purified from various human cells, as recombinant protein is synthesized and purified from *Escherichia coli* using various tags. In this study we present evidences, that yeast

Saccharomyces cerevisiae is by far better host for expression and purification of native recombinant human BiP/GRP78 protein. Main our finding – *S. cerevisiae* cells recognize and correctly process native signal sequence of human BiP/GRP78 protein consequently secreting it into the growth media – allows simple one-step purification of highly pure recombinant human BiP/GRP78 protein with yields reaching up to 10 mg/l. Further, our data shows that it is fully intact and active protein. Yeast derived human BiP/GRP78 protein possesses ATPase activity and is able to renaturate denaturated luciferase protein. In this instance *S. cerevisiae* incorporates best qualities of both human and *E. coli* cells – it produces human-like BiP/GRP78 protein herewith allowing its simple, tag-free and cost-effective purification. Thus *S. cerevisiae* cells are perfect for production of human BiP/GRP78 protein.

P14-53
Expression of ram seminal plasma proteins with sperm-preserving capacities

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The adsorption of seminal plasma proteins to the ram sperm surface partially restores the functional characteristics of damaged spermatozoa being RSVP14, RSVP20 and RSVP22 mainly responsible for this protective effect. In this study, we try three different methods to express these proteins. RNA extracted from ram seminal vesicles was employed to obtain cDNA by retrotranscription. Each sequence was amplified by PCR with specific primers, ligated with the corresponding vector and transformed in *E. coli*. Plasmids were extracted and sequenced.

Insect cell extract expression: the plasmid pF25A was used to express the proteins in a cell-free protein synthesis system prepared from insect (TNT T7). Four microgram of recombinant plasmid were added to 40 µl of TNT T7 Mix and incubated at 30°C, 4 hour.

Yeast expression: Pichia pastoris yeasts were transformed by electroporation with pPICZα recombinant vectors. After selection in YPDS plates with antibiotic the yeast were grown in BMGY medium for 24 hour and protein expression was induced during 4 days by the addition of methanol. Supernatant was checked for secreted proteins.

Escherichia coli expression: *E. coli* strain OrigamiB(DE3)pLysS was transformed with plasmids pET32a and pPSG-IBA3 and expression was induced with IPTG under different time/temperature conditions. Both the soluble and insoluble cytoplasmic fractions were analysed.

Protein expression was checked by SDS-PAGE and western blot. The proteins have been expressed in insect cell extracts and the insoluble fraction of *E. coli* but not in yeast. Studies to purify the desired proteins by nickel affinity chromatography are currently in process.

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P14-54
A biotechnological approach for the development of an enzyme-based platform with applications in the field of personalized medicine

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'Personalized medicine' is a new concept in healthcare, one aspect of which defines the specificity and dosage of drugs according to effectiveness and safety for each patient. Dosage strongly depends on the rate of metabolism which is primarily regulated by the activity of cytochrome P450. In addition to the need for a genetic characterization of the patients, there is also the necessity to determine the drug clearance properties of the polymorphic drug metabolising enzymes.

It is well-recognized that patients administered a particular drug will exhibit significant inter-individual variability in their response to treatment. Unfortunately some patients will fail to respond to the therapy entirely, while some others will suffer dose-related side effects, resulting in significant costs and fatalities. For these reasons, polymorphism in genes encoding the drug metabolizing cytochromes P450 and flavin-containing monooxygenases is a very important factor that can no longer be neglected in the development of new drugs.

Progress in the human genome analysis has recently made it possible to identify a patient's cytochromes P450 make up by genotype analysis using the AmpliChip CYP450 Test available from Roche Diagnostics. However, genotyping needs a parallel enzyme-based platform capable of rapidly measuring a drug's pharmacokinetics and clearance by the polymorphic drug metabolising enzymes typical of a given genotype, and to this date, such a platform is not available; this is the subject of the present work.

P14-55
Production of human recombinant prethrombin-1 and prethrombin-2 in *E. coli* expression system and their activation to thrombin

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The polyfunctionality of specific plasma serine protease thrombin is currently receiving increasing interest in biopharmaceutical and biomedical research and it is widely used in biotechnology and pharmaceutical industry. Optimal way to prepare pathogen-free human thrombin, with respect on strict requirements to be used as a pharmaceutical reagent applied in clinical practice is based on technics of recombinant DNA. Recombinant human thrombin has the advantage of being minimally antigenic and devoid of the risk of viral transmission in clinical applications. *E. coli* expression system is the most widely used system for expression of heterologous proteins because of its inexpensive carbon source requirements for growth, rapid biomass accumulation, ability to high-cell density accumulation and simple process scale up. In this project we have designed an expression vector and prepared a proper *E. coli* host system for human recombinant thrombin production. We have optimized the heterologous expression of prethrombin-1 and prethrombin-2, two alternative intermediate products of α-thrombin, in Erlenmeyer flask, batch and fed-bacth cultivation in fermenter. The choice of host strain, medium com-

position, temperature conditions and oxygene limitation were additional important parameters for prethrombin-1 and prethrombin-2 production in Erlenmeyer flasks and fermenter. The expressed prethrombin-1 and prethrombin-2 formed intracellular inclusion bodies from which the protein was partly refolded. In the next step we would like to activate prethrombin-1 and prethrombin-2 to active thrombin using a snake venom, ecarin.

This contribution is the result of the project implementation: 'Industrial research of new drugs based on the recombinant protein' ITMS 26240220034 supported by the Research & Development operation Programme funded by ERDF.

P14-56
Genetic expression and enzymatic activity analyses of a β-1,4 endoxylanase from *Colletotrichum lindemuthianum*

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Xylan is part of the hemicelluloses present in the secondary plant cell walls of lignified tissues and the most abundant polysaccharide in nature after cellulose. Xylan degradation by microorganisms, requires a complex of enzymes which act synergistically to convert xylan to their simpler constituents. The enzyme key to the depolymerization of the xylan is the β-1,4 endoxylanase (EC 3.2.1.8) which hydrolyses the β-1,4 binding, generating xylooligosaccharides, which in turn are hydrolyzed by β-xylosidase (EC 3.2.1.37). In addition, these enzymes and their genes have been previously cloned and characterized biochemically and molecularly in some species of fungi. In previous work we isolate and identify the cDNA for the *Clxil1* gene encoding a xylanase of the phytopathogenic fungus *Colletotrichum lindemuthianum* race 1472. In this study, we revised the genetic expression of *Clxil1* and enzymatic activity of β-1,4 endoxylanase from *C. lindemuthianum* race 1472. The genetic expression of *Clxil1* in mycelium growth on glucose don′t showed catabolite repression, and low levels of xylanase activity are detected. Furthermore, the fungus mycelium grown in xylan shows increased expression of the *Clxil1* transcript from 0 to 24 hour and in the following days (3–9 days) and substantially stimulated the increase of xylanase activity. The growth of the fungus on plant cell walls also showed a constant expression during the first hours that was higher during the following days, and the levels of xylanase activity were 77% with respect to those induced by xylan. These results suggest that *Clxil1* is transcribed in basal levels and that xylan and the cell wall increase transcription and enzymatic activity.

P14-57
Mitogen activated kinase phosphatase 2 (MKP2) regulates of vaccinia-related kinase 1 (VRK1) independent of phosphatase activity

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The vaccinia-related kinase (VRK) family is comprised of three members: VRK1, VRK2, and VRK3.

The VRK family is a class of mammalian Ser/Thr kinases that shows similarity to the B1R Ser/Thr kinase of vaccinia virus.

Among the three isoforms, VRK1 has been studied better than the others, but the functions of VRKs are still largely unknown. VRK1 can phosphorylate several transcription factors such as p53, ATF2, and c-Jun.

Since VRKs were discovered in 1997, their *in vivo* functions have remained incompletely understood. Here, we identified that MKP2, a member of the dual-specificity phosphatase (DUSP) family, is a novel binding partner of VRK1.

In a previous report, we demonstrated that VRK1 plays a crucial role in histone H3 phosphorylation to condense chromatin and cause cell cycle progression.

Therefore, we tested the possibility that MKP2 could regulate VRK1 function in histone H3 phosphorylation. We found that MKP2 specifically blocked VRK1-mediated phosphorylation of histone H3 regardless of its phosphatase activity.

However, MKP2 failed to directly act on phosphorylated histone H3, implying that blockage of histone H3 phosphorylation is due to inhibition of VRK1 by MKP2.

We also discovered that MKP2 overexpression caused a decrease in histone H3 phosphorylation and inhibition of cell cycle progression.

Together, our results demonstrate that MKP2 negatively regulates VRK1 activity on histone H3 phosphorylation and is involved in control of the cell cycle.

P14-58
Molecular phylogenetic relationship and salinity resistance of *Tetraodon* pufferfish

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Pufferfish belonging to genus *Tetraodon* form the largest group in the family Tetraodontidae. This group consists of more than 20 species with diverse distribution in fresh and brackish waters from Africa to Southeast Asia except seawater. Many of these are available in markets as aquarium fish because they are small-sized with various color patterns in their body. Furthermore, due to the smallest genome size among known vertebrates at roughly 340 million base pairs for *Tetraodon* fish, *T. nigroviridis* has been used as a model organism for comparative genomics and its draft genome sequence was published in 2004. However, little is known about phylogenetic and evolutionary relationship of *Tetraodon* species. In the present study, we performed molecular phylogenetic analysis on 17 *Tetraodon* species and 42 related species from 12 genera based on 16S rRNA and cytochrome *b* gene sequences.

Molecular phylogenetic analysis based on the sequences classified *Tetraodon* species into three groups which were well reflected by their habitats including African freshwater, Asian brackish water and Asian freshwater. Interestingly, the divergence between Asian and African species was considered to be triggered by the separation of the presumptive Pangea continent into Eurasian and African continents 60-50 million years ago. We also showed that salinity tolerance of Asian brackish water species *T. nigroviridis* was apparently higher than that of Asian freshwater species *T. cochinchinensis*, suggesting that the second specification of *Tetraodon* species occurred in the Asian group by the molecular evolution concerning osmotic regulation.

P14-59
Molecular cloning and characterization of a novel hydrolytic gene cluster from the intestinal metagenome of *Hermetia illucens*

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The larvae of the black soldier fly, *Hermetia illucens*, are voracious feeders of various organic materials among common environmental colonizer on animal wastes, and may thus be exploited as a simple system for processing daily food wastes. To identify the applicable biocatalytic genes, we constructed metagenomic fosmid libraries using the larval intestinal microbiome of the fly. The hydrolytic enzymes encoded by uncultured intestinal microorganisms were subjected to substrate hydrolysis analysis using carboxymethyl cellulose as a sole carbon source. Functional screening revealed a novel 36 295 bp gene cluster, encoding nine putative hydrolytic genes, including four endo-β-mannosidases, three β-glucosidases, a polygalacturonase, and a cellulase gene. The cluster also included additional transporter genes, and putative transcription factors. Among them, four mannosidases ranged from 1101 to 1134 bp in length with a similar pI value of 5.5 even though there were no significant pair-wise sequence similarities. One of the mannosidase genes encoded a 427 amino acid protein (designated as EM5), with a predicted molecular weight of 48.4 kDa. The encoded EM5 protein exhibited the highest sequence similarity (77%) to a hypothetical protein found in *Dysgonomonas mossii*. The purified recombinant EM5 enzyme worked optimally at 60°C and pH 7.0. The catalytic activity of EM5 was maximal with *p*-nitrophenyl-β-D-mannoside, indicating that it was a mannosidase. The presence of mannsodiase genes splitted with insertion sequences suggest that the gene cluster has acquired the specific organization by lateral gene transfer in the intestinal microbiome of *Hermetia illucens* [This work is supported by the grants from National Academy of Agricultural Science, Rural Development Administration Project No. PJ008649 and IPET Project No. 110037-03-1-HD110].

P14-60
Homologous and heterologous expression of dehydrogenases and oxidoreductases of *Ralstonia eutropha* H16

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Ralstonia eutropha has great interest for biotechnology. It can use H_2 and CO_2 as sole sources of energy and carbon in the absence of organic substrates. Furthermore this bacterium has already been applied for the production of biodegradable polyhydroxyalkanoates on an industrial scale. *R. eutropha* also serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification.

In our project we are interested in establishing specialized *R. eutropha* based cell factories by genetic engineering. The particular interest is constructing cells efficiently performing oxidoreductase reactions by overexpression of homologous and/or heterologous enzymes.

One of the main types of oxidoreductase reactions is performed by dehydrogenases, particularly alcohol dehydrogenases, which have a wide range of possible biotechnological applications. Biotransformations involving the interconversion of alcohols, aldehydes and ketones have great potential for the commercial production of pure optically active compounds and also for other processes such as the treatment of industrial effluents.

The genome of *R. eutropha* H16 contains a remarkable diversity of oxidoreducteases. A selection of alcohol dehydrogenases as well as short chain dehydrogenases of *R. eutropha* H16 was cloned and expressed in native and his-tagged versions in *Escherichia coli*. Their activity was analyzed by NAD/NADH dependent enzyme activity assays with different substrates. Currently we are working on homologous expression of selected alcohol and short chain dehydrogenases in *R. eutropha* H16 and their functional analysis.

P14-61
Cloning and expression of carbon cycle relevant enzymes of *Ralstonia eutropha* H16

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Ralstonia eutropha is a Gram-negative, strictly respiratory facultative chemolithoautotrophic bacterium which can use hydrogen and carbon dioxide as sole sources of energy and carbon in the absence of organic substrates. It has attracted great interest for biotechnology for its ability to degrade a large list of chloroaromatic compounds and chemically related pollutants. Moreover the production of biodegradable polymer polyhydroxyalkanoates on an industrial scale has already been applied [1]. *R. eutropha* serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification.

In our project the main objective is the cloning of different enzymes, like carbonic anhydrases and carboxylases, which allow the organism to fixate carbon dioxide. Important enzymes for the carbon dioxide fixation under lithoautotrophic growth conditions are Rubisco as well as PEP-carboxylase and pyruvate-carboxylase. These enzymes are not able to directly use carbon dioxide as substrate. Carbonic anhydrases are responsible for the conversion of CO_2 to HCO_3^-, which can be used by these carboxylases [2, 3]. The overall aim of the study is the overexpression of carbonic anhydrases and carboxylases to provide a growth benefit to *Ralstonia eutropha* H16 under lithoautotrophic conditions.

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P14-62
Molecular mechanism of co-super-production system of lipase and surface-active protein from pseudomonas-like bacteria

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Stable and high stereo-selective lipase and esterase have attracted much attention from viewpoints of industrial usage. In the course of our studies searching for efficient inducers for lipase, among various additives, we have found that fatty alcohols (stearyl alcohol, etc.) and polyoxyethylene (n) alkylethers (POE 20 oleylether, etc) act as the most effective super-inducers for induction of thermo-stable and stereo-selective lipase production by several *Pseudomonas*-like bacteria.[1] The addition of fatty alcohols brought about more than several hundred-fold enhancement of the lipase activity compared to the case with no additive. This means several dozen-fold enhancement of lipase activity compared with olive oil grown case. Lipase production at the gram-per-liter scale and purification has been capable. We also found that when several *Pseudomonas*-like bacteria were grown on fatty alcohols, not only lipases but a large amount of an extracellular 15 kD protein (P15) was strongly induced. P15 was identified to be a surface-active agent from the following facts: Purified P15 reduced surface tension of water and possessed n-alkane emulsifying activity. Both lipase activity and the amount of lipase gene transcript in P15 gene deletion mutant-strain were remarkably reduced compared with those in wild-type strain, so much so that P15 seems to be concerned in the lipase super induction by fatty alcohols. P15 gene seems to be under a different transcriptional regulation from that of lipase gene as a result of 5′-upstream region analysis. We propose a model of lipase super-production system as follows: uptake of a small amount of lipase super-inducer, induction of P15 protein and secretion, emulsification of stearyl alcohol by P15 protein, uptake of a large amount of lipase super-inducer, and strong induction of lipase and secretion.

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P14-63
Characterization of a yeast mutant depleted for the hypusine posttranslational modification

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In eukaryotic cells, deoxyhypusine synthase (*Dys1* in *S. cerevisiae*) is an essential enzyme responsible for the unusual posttranslational modification of the putative archaeal/eukaryotic translation initiation factor 5A (aIF5A/eIF5A). eIF5A is an essential and highly conserved protein present in all organisms from archaea to mammals. This factor has recently been described as playing a role in the elongation step of translation and is the only protein known to contain the unique and essential amino acid hypusine. The hypusine residue is formed by a two-step modification named hypusination catalyzed by the enzymes deoxyhypusine synthase and deoxyhypusine hydroxylase (Lia1 in *S. cerevisiae*). Despite the fact that hypusine formation involves two different enzymatic steps, in *S. cerevisiae* only the gene for deoxyhypusine synthase is essential for cellular growth. Furthermore, inhibition of deoxyhipusine synthase results in cellular growth arrest and alterations in tumor cell proliferation and differentiation. Due to its essentiality for eIF5A function and in an attempt to identify the biological function of the hypusine residue, we generated the first conditional mutant of the deoxyhypusine synthase gene in yeast, designated *dys1-1*. The *dys1-1* mutant shows a dramatic decrease in Dys1 protein levels leading to very low levels of hypusinated eIF5A. Furthermore, this mutant shows an increased cell size, grows only in the presence of an osmotic stabilizer and shows a reduction on growth rate, which is not related to massive cell lysis. Finally, the *dys1-1* mutant demonstrates a reduction in the total protein synthesis and a defect in the elongation step of translation corroborating with the essentiality of this posttranslational modification of eIF5A. These results are in agreement with those previously shown for eIF5A mutants. Further studies using the *dys1-1* mutant will contribute to clarify the impact of the hypusine residue in the function of eIF5A. Supported by: FAPESP, CNPq and PADC.

P14-64
Subcellular localization and transport efficiency of protein sorting signal sequence

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Bio-synthesized proteins are localized to particular organelles in order to carry out their biological functions. Organelle sorting sequences including signal-peptides, mitochondrial targeting sequences, nuclear localization sequences are well known to play an essential role in transporting proteins to the appropriate organelles. However, there still remain several unsolved questions regarding the molecular mechanism of protein.

Signal-peptide is a sequence which consists of about twenty-five amino acids which are responsible for protein transport to the Endoplasmic Reticulum (ER). After being localized in the ER, the main part of the protein is detached from the signal-peptide and becomes a mature-form protein attached by modification molecules including oligosaccharides, lipids and glycolipids. However, why various sequence patterns exist in signal-peptides is still unclear.

In this study, the relation between subcellular localization, organelle transport efficiency and physicochemical properties of amino acids which consist of signal-peptides were evaluated using microscopic study and immunoassay by examining the fusion of green fluorescence proteins (GFPs) to signal-peptide. Signal-peptides derived from native GPI-anchored proteins and several artificial sequences like signal-peptides were introduced into GFP. GFP fluorescence in HeLa cells transfected by the expression vector for signal-peptide-GFP fusion protein was observed by a confocal laser scanning microscope. Subcellular localizations of signal-peptide-GFP fusion proteins were examined by comparison with the fluorescence image using organelle markers. Organelle transport efficiencies were evaluated quantitatively by the western blotting of cell fractions.

P14-65
Microsatellite analysis of Kangal shepherd dogs in Turkey, Uzbekistan, Ajerbaijan and Iran

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Microsatellite markers are widely used in population genetics studies due to their high polymorphism and versatility. A total of 10 microsatellites were used to investigate genetic diversities of four Kangal Shepherd dogs in Turkey, Uzbekistan, Ajerbaijan and Iran. Blood samples were collected from Kangal dogs in Turkey (n = 31), Ajerbaijan (n = 29), Uzbekistan (n = 30) and Iran (n = 37) and DNAs were isolated. The amplified Polymerase Chain Reaction (PCR) products were separated by capillary electrophoresis and alleles were determined. General population parameters were calculated and phylogenetic trees were drawn. Allele numbers were ranged 12.5 and 15.2. Average observed (Ho) and expected (He) heterozygosities were observed as 0.610–0.667 and 0.783 and 0.828, respectively. A statistically significant ($p < 0.001$) Fst value was observed in F-statistics of the molecular data. In factorial correspondence analysis (FCA) and neighbor-joining tree, Kangal shepherd dogs in Turkey were positioned differently from the other Kangal populations.

P14-66
Genetic characterization of Akbas shepherd dogs in Turkey, Uzbekistan and Iran using STR markers

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The objective of this study was genetic characterization of Akbas dogs located in Turkey, Uzbekistan and Iran using 10 microsatellites (FH2011, FH2161, PEZ5, FHC2010, FH2324, FH2247, PEZ6, PEZ11, PEZ8 and FHC2079). A total of 98 blood samples were collected and DNAs were isolated. Marker loci were amplified by Polymerase Chain Reaction (PCR) and alleles were determined by fragment analysis. Different statistical methods were used for investigation of diversity within and between the Akbas populations. Mean allele numbers were 12.3 (Uzbekistan), 14.7 (Iran) and 13.2 (Turkey). Average observed (Ho) and expected (He) heterozygosities were ranged from 0.597 to 0.717 and from 0.777 to 0.823, respectively. The general Fst (0.04375) value was significant ($p < 0.001$). In phylogenetic trees, Akbas dogs in Turkey and Uzbekistan were clustered together. In Factorial Correspondence Analysis (FCA) analysis, all Akbas population were positioned separately, however Akbas' in Turkey and Uzbekistan illustrated a relatively close localization.

P14r-67
Cloning and expression of three GAPDHs from *Pseudomonas syringae* pv Tomato: biotechnological implications

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The gammaproteobacterium *Pseudomonas syringae* pv. Tomato DC300 is the causal agent of bacterial speck, a common disease of tomato characterized by small brown-black specks, 1–3 mm in diameter, surrounded by chlorotic yellow halos. These lesions constitute a severe blemish on fruit for fresh market and yield potential. Up to now, the mode of infection of *P. syringae* is not well understood, but according to molecular biology, genomic and proteomic data, this phytopathogenic bacterium produces a number of proteins that may promote infection and draw nutrients from the tomato plant. Among these proteins, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a major enzyme of central C metabolism, can play a role in the infection process, as a potential surface antigen and virulence factor.

Whole-genome sequence analysis of *P. syringae* pv. Tomato DC300 strain, allowed us to identify three paralogous *gap* genes encoding GAPDHs with distinct predicted molecular and catalytic features, one of them being expected to be actively involved in the infection process. By using genome bioinformatics data, as well as alignments of both DNA and deduced protein sequences of the three GAPDHs of *P. syringae*, we constructed sets of specific primers in order to amplify the corresponding *gap* genes by PCR reactions. The PCR-amplified ORFs of paralogous *gap* genes of *P. syringae* were cloned on one step at pet21a with Tag-system and over-expressed in *E. coli*. The recombinant proteins were purified to study their molecular and catalytic properties, and to eventually generate monospecific antibodies. Our preliminary results show that these GAPDH isoforms have diverse molecular masses and unusual NAD(P)-cofactor specificities, suggesting specific metabolic roles.

The possible association of these bacterial GADPHs with cell outer-membranes, a scenario with significant biotechnological implications, is currently under investigation by using immuno-(cito)chemical and other experimental approaches.

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P14m-68
The *Pseudomonas syringae* pv tomato GAPDH genes are differentially up-expressed under infective conditions

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In plant health as human or animal health, we are witnessing the arrival of new diseases, so-called emerging. One of these is bacte-

rial speck, common disease of tomato caused by *Pseudomonas syringae* pv. Tomato, but the molecular mechanism of this plant infection, is not yet well-understood. According to Molecular Biology and Proteomics data, *P. syringae* produces a number of proteins that promote infection and draw nutrients in the tomato plant. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key glycolytic enzyme, is a likely candidate for infection-related bacterial protein. By using whole-genome sequence and proteomic data we found that *Pseudomonas syringae* pv. Tomato DC300 strain posses three paralogous *gap* genes encoding GAPDH proteins with different predicted molecular sizes and metabolic functions one of them being probably involved in non-enzymatic functions. To understand the real impact of the three *gap* genes on pseudomonas syringe virulence, in the current study we proceed to analyze the expression level of each paralogous gene. To do that we infected the plant of tomato with inoculums from *Pseudomonas Syringae* pv tomato, and extracted total mRNA. The expression level of each individual bacterial *gap* gene was subsequently determined by One-Step RT-PCR looking for possible differences in mRNA expression between cells under infective and normal physiological status. It was found that in the infective state of the bacterium, there is a clear up-expression of transcripts of the three gap genes in comparison with the normal state. Moreover, there was also difference in expression of each gene in this state, suggesting specific functional roles under physiological condition. In the end of this work we determine which GAPDH of the three seems more involved in the virulence of Pseudomonas Syringae Support of Collaborative grant A1/043076/11 and A/030965/10. of the Spanish AECID (MAEC) is acknowledged.

P14-69
Expression levels of metabolizing and xenobiotic efflux proteins are altered by antibiotic treatments in salmon cell lines

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The greatest economic losses in the Chilean salmon aquaculture have been produced by infectious pathologies; principally bacterial and virus infections. The occurrence of different bacterial infections has triggered a massive use of several antibiotics, which are also widely used in most of the diverse productive sectors of the Chilean aquaculture. Nonetheless, the effects of these drugs in the general physiology of exposed fish still are just beginning to be studied. The effect of antibiotics commonly used in salmon farming, such as flumequine, florfenicol, oxytetracycline and oxolinic acid, on the expression levels of proteins involved in metabolism and xenobiotic efflux (MDRs) in SHK1 and CHSE cells cultured *in vitro* was evaluated. The expression levels of proteins involved in drug metabolism (CYP1A, CYP3A, FMO and GST) and proteins involved in xenobiotic efflux (Pgp and MRP1), were measured by Western blot after antibiotic treatments, at a concentration of 10 μg/ml in assays of 24, 48 and 72 hours. Cell viability was also measured by MTT assays. The results obtained showed significant fluctuations with respect to control in the levels of expression in the most proteins analyzed as response to antibiotic treatments, while no effect on cell viability was observed. These results show that in these salmon cell lines, the treatment with antibiotics changes the expression levels of protein metabolism and of drug resistance proteins, which may be associated with the induction of drug-drug interactions and development of drug resistance phenomena for subsequent drug therapy. This information allows a deeper understanding of the mechanisms involved in cell metabolism and elimination of drugs in marine organisms whose production belongs to one of the most important economic sectors in our country. This work was supported by Grants 1120903 and D08i1096 from the Fondo Nacional de Desarrollo Científico and Tecnológico de Chile (FONDECYT) and Fondo de Fomento al Desarrollo Científico (FONDEF), respectively; and Dirección de Investigación y Desarrollo from Universidad Austral de Chile (DID-UACh).

P14-70
Usage of silica coated magnetic nanoparticles and SERS nanotags for gene detection

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Surface-enhanced Raman scattering (SERS) spectroscopy has attracted considerable interest for biomedical applications such as DNA detection. Magnetic nanoparticles have also been applied to the similar areas such as immunoassay, bioseparation and biosensors. Since the silica shells usually possess the properties of biocompatibility and easy functionalization, magnetic nanoparticles consisting of cores of superparamagnetic particles and silica shells are preferred for practical applications. In this study, both silica coated magnetic Fe_3O_4 and silver nanotags were used to establish a DNA hybridization assay for SERS detection of DNA oligonucleotides.Magnetic nanoparticles were synthesized by thermal decomposition method in order to obtain small (4–6 nm) and monodisperse Fe_3O_4 nanoparticles by using $Fe(acac)_3$ as precursor, benzylether as a solvent and oleylamine as a reducing agent.Reverse micro emulsion method was used for silica coating of Fe_3O_4 nanoparticles and silica layer thickness was adjusted approximately 4–5 nm.Magnetic nanoparticles are transferred cyclohexane, and polyoxyethylene(5)nonylphenyl ether, tetraethylorthosilicate and ammonium hydroxide were added to form a reverse microemulsion solution. Surface of silica coated Fe_3O_4 nanoparticles were modified by amino group in order to provide attachment of probe archaea proteasome gene sequences onto the silica surface. Characterizations of nanoparticles were done by EDX, SERS, UV-vis, Fourier infrared spectrometry and SEM. The synthesis of Ag/SiO_2 nanoparticle-based SERS tags was also accomplished by using reverse micelle technology.Target archaea proteasome gene sequences were immobilized to the surface of silica coated Fe_3O_4 nanoparticle through amino groups. The archaea proteasome gene probe was labeled with SERS nanotags. After hybridization of target and probe archaea proteasome gene sequences, these hybridization complexes were separated from solutions by applying magnetic field. Raman activity of the labels was measured by SERS spectroscopy.

P14-71
Application of SERS nanotag labeled probes for DNA studies

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The ability of insertion of metal nanoparticles into biological systems have many advantages in biology and biomedicine. However, there is an important limitation for the detection of biomolecules. In that respect, Surface Enhanced -Raman Scattering (SERS) is one of the useful technique for detection of biomolecules.

The aim here is to prepare SERS nanotags, which include Raman enhancing metal nanoparticle, a Raman reporter molecule attached to the surface of the nanoparticle, and an encapsulant. The role of the encapsulant is to surround not only metal nanoparticle but also the Raman active molecule as well.

For this, SERS is an useful technique for the detection of simultaneous multi component detection for complex biological matrices. Two different approaches, namely Stöber method and reverse microemulsion system, were used for coating a silica layer onto the metal nanoparticles. Attachment of biological active ligands onto the silica-coated SERS tags provide a variety of applications. For example, attachment of nucleotides after surface modification allows SERS nanotags act as biosensors to detect some biological activities such as hybridization of DNA. On the other hand, embedding of Raman reporter molecules into the silica layer of metal nanoparticles allows signal enhancement in Raman Spectroscopy.

In this study, Ag core nanostructures for SERS nanotags were synthesized by chemical reduction method, and then synthesized nanostructures were coated via Stöber method and modified reverse microemulsion method for dye labelling. In both procedures, silica layer formation depends on hydrolysis and condensation of a silica precursor, tetraethoxysilane (TEOS). Raman reporter molecule was embedded into silica at this step. With the addition of water to silane solution under acidic, basic and neutral conditions, hydrolysis step starts. After the surface modification of amino groups onto silica surface, immobilization of archaeal citrate synthase gene probe was achieved. Later on, hybridization experiments were carried out and SERS active label peak was followed.

P14-72
Determination of proteasome activity using surface enhanced raman scattering (SERS) spectroscopy

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Detection of increased or decreased level of proteasome activity is an important diagnostic indication for pathological state of the cell, and may have prognostic significance, as well. The proteasomes are often overexpressed in many cancer cells; for example abnormally high expression of proteasomes have been found in human leukaemia cells, renal cancer cells and in breast cancer cell lines. On the other hand, in age-related decrease in the expression of proteasomes is observed in pathologies such as neurodegenerative and cardiac diseases. In this study, we aimed at specific detection of levels of proteasome gene expression in patients as an alternative to available enzyme activity measurement methods. It is known that, nanotechnology have permitted the development of novel assays for molecular diagnosis. Surface enhanced Raman scattering-SERS spectroscopic technique was used for quantitative analysis of proteasomes. In SERS method, SERGen probes were designed using specific proteasome gene sequences which were labeled with Raman active dyes and single-dye SERS nano tags. Target gene sequences were immobilized either to the surface of a gold plate or on silica coated Fe_3O_4 nanoparticles. The results of hybridization and immuno detection assays indicated a linear correlation between the SERS signal intensity and SERS probe concentration. Therefore, this SERS system can be suitable for quantification of the circulating proteasome levels and our research to adapt it for clinical applications is underway.

P14-73
mRNA triphosphatase assays

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The disease burden of malaria is heaviest in young children in sub-Saharan Africa. The spread of resistance to existing anti-malarial drugs means that it is imperative that new treatments to control the disease are developed. Malaria is a major focus of research within the DDW (Diseases of the Developing World) with its base at Tres Cantos.

The 5′ terminus of eukaryotic mRNA is capped by a 7-methyl guanosine linked by a 5′-5′ triphosphate bridge to the first nucleotide of the transcript. The capping process involves the concerted action of three enzymes, first of them being RNA triphosphatase, which hydrolyses the g phosphate of the triphosphate-terminal RNA. Due to the central role of the mRNA cap in Plasmodium gene expression, inhibiting the triphosphatase activity could provide an anti-malarial drug.

To identify inhibitors of *Plasmodium falciparum* triphosphatase a high throughput screening has been run. Hits have been characterized against *P. vivax* and Human triphosphatases as well as with citotoxicity assays. In this poster is presented the integrative set of assays that are allowing the identification of specific and selective inhibitors of the mRNA capping process in *Plasmodium*.

P15 – Molecular Parasitology

P15-1
Quantification of the effect of potential virus inhibitors

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Measuring the degree of virus inhibition by potential effectors is now recognized as primary task under the development of antiviral drugs. Here, we describe a highly sensitive method that allows the correct quantification of inhibition effect with a high degree of accuracy directly at the molecular level. The protocol involves two stages, namely serological virus titration in comparison with the same procedure for virus-effector mixture. Owing to the robustness of the analysis this assay can be performed on crude cellular and plant extracts, and therefore it may be suitable for the routine analysis of clinical samples or in the field. The efficiency of the approach to the quantification of the inhibition effect of polysaccharide glucuronoxylomannan (GXM) on the infection efficiency of the tobacco mosaic virus (TMV) was investigated using advanced serological approaches based on label-free surface plasmon resonance technique. It was shown that GXM drastically decrease efficiency of TMV infection by blocking up to 70% of virus shell. The obtained results are in conformity with the method of indicator plant infection. A simple analytical model for the analysis of the processes in an virus system is proposed and discussed in details.

P15-2
Removal of introns from *Theileria annulata* lactate dehydrogenase gene by genetic engineering methods opens a route to the expression of the protein towards design of new anti-theilerial drugs

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Lactate dehydrogenase gene from the *Theileria annulata*, the causing parasite of theileriosis in mainly cattle, has been cloned in our previous studies and results showed the presence of two introns in the sequence. In the present study, these two introns were removed from the gene by site directed mutagenesis, because of the lack of the cDNA, to enable the expression of the protein in an appropriate system. The site directed mutagenesis study was designed to remove both introns before insertion of the gene in to the expression vector pKK 223-3. After the removal of the introns, full length PCR product and the expression vector were digested with the appropriate restriction endonucleases and then transformed in to the *E. coli* cells following the ligation. Expression of the protein by applying this strategy opened a route to conduct the study on purification and the kinetic and structural analysis of the protein towards structure based drug design studies against the parasite.

P15r-3
Interaction of the *Leishmania* poly(A)-binding proteins (PABPs) with the host immune system

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Gene expression regulation in *Leishmania* has been related to post-transcriptional events. The initiation of protein translation is an important checkpoint. This process depends on the interaction of a group of proteins known as tranlation initiation factors, including poly (A)-binding proteins (PABPs). On our research, the antigenicity and immunogenicity of the *Leishmania infantum* PABPs have been studied. With this purpose, three genes encoding different *L. infantum* PABPs were identified in the genome database using as query the sequences of the previously described *L. major* PABP variants. The corresponding *L. infantum* proteins were expressed and purified as recombinant products. Antigenicity of the three PABPs has been studied using sera from human patients infected with different *Leishmania* species. In addition, we have analysed the interactions of the *L. infantum* PABPs and the host immune system using two experimental models of murine cutaneous leishmaniasis due to *L. major* infection (BALB/c susceptible mice and C57BL/6 resistant mice). Humoral and cellular responses observed in the murine models have revealed interesting immunological properties of the parasite PABPs.

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P15-4
Trypanosomal uracil-DNA glycosylase is involved in maintenance of genome stability and counteracts DNA damage induced by nitric oxide

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Cells contain low amounts of uracil in DNA which can be the result of dUTP misincorporation during replication or cytosine deamination. Elimination of uracil in the base excision repair pathway yields an abasic site, which is potentially mutagenic unless repaired. Uracil-DNA glycosylase (UNG) is the first enzyme of the uracil excision repair pathway and also appears to play an important role in the repair of DNA lesions derived from oxidative stress.

The *Trypanosoma brucei* genome presents a single UNG responsible for removal of uracil from DNA. This parasite has to resist the oxidative and nitrosative damage generated as a consequence of the genotoxic host immune response. Hence, adaptation to oxidative stress is critical for preserving cellular viability and virulence *in vivo*. We show that defective elimination of uracil via the base excision repair pathway gives rise to hypersensitivity of *T. brucei* bloodstream forms to nitric oxide and hydrogen peroxide and UNG defective cells exhibit reduced infectivity

in vivo. However, we find that exposure of mutant cells to NO prior to infection increases the expression of genes involved in thiol metabolism and increases parasite virulence. This effect was observed after exposure to NO both *in vitro* and *in vivo* thus demonstrating that reduced infectivity due to defective uracil elimination can be overcome by the up-regulation of enzymes involved in antioxidant defence and that NO can induce this protective response.

P15-5
New insights into the expression profiles of protein phosphatases 1 (PP1) in *Leishmania infantum*

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The protozoan parasite *Leishmania infantum* is the causative agent of visceral leishmaniasis in the Mediterranean basin, where domestic dogs are the main reservoir. The promastigote and the amastigote stages alternate in its heteroxene life cycle. The signalling pathways that control these differentation events and cell cycle as well as their connection to gene expression regulation have not been characterised so far. The family of serine/threonine protein phosphatases 1 (PP1) is essential in regulating diverse celular processes and is highly conserved among the eukaryotic organisms. The *L. infantum* genome contains at least seven catalytic and one regulatory PP1 subunit genes according to the GeneDB annotations. The alignment of the PP1 catalytic subunits (cPP1) of *L. infantum* have revealed that they only differ considerably in their amino terminal regions, which could be related to substrate specificities and interaction with regulatory proteins. In fact, the predicted secondary structures of the *L. infantum* cPP1 proteins do not significantly differ from the human orthologues. Interestingly, their differential expression profiles are different between the main life cycle stages. Several experiments based on custom *L. infantum* shotgun DNA microarrays have shown that the cPP1 LinJ.34.0840 is over-expressed in metacyclic promastigotes from the anterior gut of the insect vector *Phlebotomus perniciosus*, whereas the expression profiles of the cPP1 LinJ.34.0820 or LinJ.34.0830 are the opposite. Moreover, the cPP1 LinJ.15.0240 is up-regulated in logarithmic phase promastigotes. Additional studies would reveal the relation between these changes in gene expression and the association of these cPP1 with the main regulatory proteins.

P15-6
Comparative proteome analysis of the motile stages of the trypanosomatid parasites *Leishmania amazonensis* and *Crithidia fasciculata*

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Leishmania amazonensis causes human cutaneous leishmaniasis in the New World. Wild rodents are the reservoirs of this species closely related to *L. mexicana*. The promastigote stage develops inside the gut of the insect vector of the genus *Lutzomyia*, whereas amastigotes differentiate inside phagocytes of the mammalian host. *Crithidia fasciculata* is not pathogenic for mammals, as its life cycle is also dimorphic but monoxene: amastigotes are not intracellular and stick to the surface of the gut of several species of insects; the motile stage is the choanomastigote, which is transmitted via faeces mainly to larvae.

The 2D electrophoresis-based proteome analyses of the respective motile stages of both parasites were performed from triplicate axenic cultures. First, total protein extracts were prepared daily, then precipitated with TCA and quantified by the BCA method. Isoelectrofocusing of 50 μg of each extract was carried out in a pH 3–10 non-linear gradient. The 2DE gels were stained with SYPRO Ruby and analyzed with the PD-QUEST software. The normalization algorithm was total quantity in valid spots and significant differences were inferred by the paired *t*-test. Protein identification was carried out by MALDITOF/TOF mass spectrometry. Similar expression profiles consisting of up-regulation in logarithmic phase of the respective orthologue proteins LACK and CACK (analogues of activated protein kinase C receptors) were found, as well as for the respective C2 calpain-like cysteine peptidases, tryparedoxin peroxidases and hsp60 chaperonins. On the other hand, several proteins are differentially regulated only in *L. amazonensis,* such a peroxidoxin up-regulated in logarithmic phase and a putative endoribonuclease L-PSP (pb5) in stationary phase. It is remarkable that no coincidence has been found in over-expressed proteins in stationary phase between the respective motile stages. This is indicative substantial differences in the proteome profiles of infective parasites of both genera towards infection of a new host.

P15-7
Heat shock protein 83 gene analysis in *Leishmania* genus

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Human leishmaniasis is a group of diseases caused by species of the flagellated protozoan parasites that belong to the *Leishmania* genus. There are two main clinical manifestations cutaneous and visceral leishmaniasis. The cutaneous form affect skin and/or mucous membranes, and is characterized by the presence of single or multiple painless lesion, nodular lesions spread over the body, or mucocutaneous lesions that affect the nasopharyngeal region. Like other parasites, some *Leishmania* species coexist in the same geographical areas, involving in some cases the same hosts, reservoirs or vectors.

The gold standard identification technique is MLEE. However several molecular markers have been described and used. We report here the reach of hsp83 gene for identification of *Leishmania* species and for the contribution in the discussion of general taxonomy consensus of this genus.

The method used was RFLP and sequencing. Six different enzymes were analyzed in seven species within the genus. The whole gene was cutted in two fragments one of 1100 pb and another one of 944 pb, both of them were analyzed by RFLP. Moreover the fragment of 944 pb was sequenced and analyzed with Mega software.

The 1100 pb fragment has a high conserved sequence and did not show differences between species. The 944 pb analyzed by RFLP was able to differentiate between subgenus Leishmania and Viannia. L. (V.) *lainsoni* showed a different pattern.

The sequence of 944 pb of hsp83 gene, formed in Leishmania genus, a monophyletic group with two well differentiated subgen-

era. Within subgenera Viannia, L. (V.) *lainsoni* form one independent cluster. L. (V.) *braziliensis* and L. (V.) *guyanensis* are closer than L. (V.) *lainsoni*.

P15r-8
Role of *Leishmania infantum* DNA polymerase theta in translesion synthesis

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Leishmania infantum is the etiological agent of zoonotic visceral leishmaniasis in Mediterranean areas and domestic dogs are the main reservoir. In order to protect both genomic and kinetoplast DNA from mutations, Leishmania requires specific DNA repair pathways and proteins. One of them is DNA polymerase theta (Polθ), not yet described in Leishmania. Polθ is an A-family polymerase that in humans is related to translesion synthesis, bypassing blocking lesions such as abasic (AP) sites and thymine glycols. LiPolθ expressed *E. coli* was shown be an active polymerase using an *in vitro* template/primer extension assay, with either magnesium or manganese as valid metal cofactor, and behaving as a distributive DNA polymerase. LiPolθ (130 kDa), like human Polθ, tends to stall after incorporation at template C. Moreover, LiPolθ was capable to tolerate AP sites via a misalignment mechanism, resulting in a -1 deletion during extension. This behaviour is different than that reported for hPolθ, which incorporates a nucleotide in front of the lesion. We next demonstrated the ability of LiPolθ to bypass oxidative lesions as 8oxodG in the template. LiPolθ 'copied' the lesion, as it preferentially inserted a correct dCMP opposite 8oxodG. In the presence of manganese LiPolθ activity rose and the protein was also able to elongate the 8oxodG:dCMP pair, thus performing an error-free tolerance of this lesion.

Transfected Leishmanias overexpressing LiPolθ were used to evaluate *in vivo* the impact of this enzyme in damage tolerance, by using oxidative agents as H_2O_2. Overexpression of LiPolθ made Leishmanias more resistant to H_2O_2 compared to the control parasites. This LiPolθ ability could have a key role during the first steps of Leishmania infection where oxidative stress is the main defense mechanism that phagocytic cells use to kill the parasites.

P15-9
Double-stranded RNA nanoparticles directed against malarial topoisomerase II inhibits growth of *Plasmodium falciparum*

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The development of new effective antimalarial agents is urgently needed due to drug resistance of the most virulent human parasite *Plasmodium falciparum*. We have employed a long double-stranded (ds)RNA targeting the coding region of *P. falciparum* topoisomearse II gene (*PfTOP2*) that is complexed with chitosan nanoparticles to interfere with the cognate mRNA expression and determined the effect on *P. falciparum* growth in culture. Topoisomerase II is a DNA replicating enzyme essential for parasite growth. The chitosan/dsRNA nanoparticles were 100-200 nm in size with positve surface charged. Exposure of ring stage-infected erythrocytes to 10 μg/ml *PfTOP2* chitosan/dsRNA nanoparticles for 48 hour resulted in 72% growth inhibition compared with 41% inhibition using an equivalent amount of free *PfTOP2* dsRNA or 12% with unrelated shrimp yellow head virus chitosan/dsRNA nanoparticles. A decrease in parasite growth correlated with a decrease in levels of *PfTOP2* mRNA by RT-PCR analysis. The inhibition was shown to occur during maturation stages with optimal inhibition being detected after 36 hour. These results lend support to the feasibility of developing biodegradable cationic nanoparticles, such as chitosan, as a delivery system for nucleic acid-based therapeutics in malaria chemotherapy.

P15-10
Evidence of the formation of RNA granules in *Leishmania infantum*

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Parasites of the genus *Leishmania* are protozoa that can cause serious illnesses, but have also been considered as attractive models for studying molecular mechanisms. Throughout its life cycle, they undergo different stress situations due to sudden changes in temperature, lack of nutrients or living in oxidant or acidic conditions, such as during the transfer of the parasite from the invertebrate host (*Phlebotome*) to the vertebrate host or within macrophages.

In eukaryotic cells under conditions of cellular stress, there are some processes which include the formation of RNA granules. Untranslated mRNAs together with proteins involved in splicing, transcription, adhesion, signaling and development are kidnapped and stored in these granules. It has been shown to play an important role as modulators of gene expression in post-transcriptional processes and epigenetic. Its formation has been observed in yeasts, protozoa and metazoa, including trypanosomatids as *Trypanosoma brucei* and *Trypanosoma cruzi*.

In this paper we demonstrate the presence of RNA granules in *Leishmania infantum*. We established situations of heat shock caused by the temperature change of the culture from 27 to 41°C for 3 hours. The use of anti-TIA-1 antibody has allowed us to confirm the formation of these granules in these conditions.

Furthermore, we also confirmed its formation in situations of oxidative stress by employing sodium arsenite at different concentrations, also being able to observe the formation of these granules.

P15-11
Localization and functional characterization of farnesyl pyrophosphate synthase (FPPs) involved in the isoprenoid pathway during intra-erythrocytic cycle of *Plasmodium falciparum*

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Many types of isoprenoids are essential components of the cellular machinery of many organisms. All isoprenoids derive from a

common precursor, the isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). The enzyme farnesyl diphosphate synthase (FPPs) catalyzes the condensation of IPP with geranyl diphosphate (GPP) to form the farnesyl diphosphate (FPP), a substrate which catalyzes the first step in the biosynthesis of ubiquinone, dolichol and proteins isoprenylation. FPP is condensed with a further molecule of IPP by enzyme geranylgeranyl diphosphate synthase (GGPPs) to form the geranylgeranyl diphosphate (GGPP), also essential for protein isoprenylation and carotenoid biosynthesis.

Recently our group characterized the FPPs in *P. falciparum*, showing the bifunctionality of the enzyme, identifying the formation of the FPP and GGPP.

The sequence of DNA (~0.8 kb) corresponding to the C-terminus of FPPs was cloned in the vector pTEX150-HA/Stre3 containing the epitope of hemagglutinin protein (HA), generating the vector pFPPs-HA. Similarly was constructed a vector for integration where the protein is expressed in fusion with HA-DD24. This domain (DD) has a site for a ligand (Shld1) and is structured to be unstable in the absence of ligand, therefore, the fusion protein is degraded.

Integration of pFPPs-HA in the genome of *P. falciparum* was confirmed by PCR, showing the fragment of the expected size of FPPs (2.2 kb) fused to the HA tag. The expression of the protein with the expected size of 47 kDa fused the HA was observed by western blot. We showed, using this transfected strain, that the FPPs is expressed constitutively, i.e. during all intra-erythrocytic cycle stages of *P. falciparum* and it was that this enzyme is located in the cytosol of parasite.

We are conducting experiments to determine the importance of FPPs during the intra-erythrocytic development of *P. falciparum* by regulating the expression of this enzyme using the strain pFPPs-HA-DD24. Results generated will help understand more about new targets for drugs.

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P15r-12
Nanobody-coated nanoparticles strongly reduce trypanocidal pentamidine curative dose and defeat drug resistance

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African trypanosomiasis (HAT) is a vector-borne disease that prospers in impoverished rural parts of sub-Saharan Africa, where millions of people are at risk of infection. It is caused by the flagellated protozoa *Trypanosoma brucei* and is transmitted by tsetse flies of the genus *Glossina*. Current chemotherapy relies on only four drugs that have many limitations, ranging from problems with oral absorption, acute toxicities and the emergence of trypanosomal resistance, which is a major concern owing to the absence of a vaccine or therapeutic alternatives. All the resistance mechanisms known are associated with loss of surface transporters.

Nanobodies (Nbs) are small antibody fragments, with unique antigen recognition properties, derived from conventional camelid antibodies through recombinant gene technology that can be used to target biological active components. In this context, we have prepared pentamidine loaded nanoparticles of chitosan polymer coated by a specific nanobody that target the surface of *T. brucei*. The nanobody-coated nanoparticles bind to the trypanosome surface and the drug is taken up by endocytosis instead its classical membrane transporters.

We next determined whether the new formulation were more efficient than pentamidine alone in killing trypanosomes. *In vitro* studies revealed that the IC_{50} of pentamidine loaded in nanobody-coated PEGlycated chitosan nanoparticles was 10 fold lower than pentamidine alone.

An *in vivo* experiment in murine model of the acute phase of African trypanosomiasis determined that the curative dose of pentamidine loaded in Nb-chitosan nanoparticles was 100 fold lower than pentamidine alone.

A pentamidine resistant cell line was obtained by growing the parasites in increasing concentration of drug during 6 months. Genetic and functional assays revealed that the resistance mechanism to pentamidine of this cell line was due to mutation in aquaglyceroporin 2, a cell surface transporter. An *in vitro* study showed that this cell line was not longer resistant to pentamidine loaded nanobody-coated nanoparticles.

P15-13
Expression and purification of recombinant fragment *Plasmodium falciparum* EBA-140 ligand binding region

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The EBA-140 ligand is a member of *Plasmodium falciparum* DBL protein family responsible for human erythrocyte binding. It shares structural homology with *P. falciparum* EBA-175 antigen – a ligand for glycophorin A on human red blood cells. The conserved binding region of each member of *P. falciparum* DBL protein family consists of two homologous domains F1 and F2. The F2 domain seems to be more important for receptor binding.

We have cloned F2 domain of EBA-140 ligand from genomic DNA of *P. falciparum* Dd2 clone. Expression of the recombinant form of F2 domain in fusion with MBP protein was carried out in bacteria (*E coli, Rosetta Gami*) using pMALc2x expression vector (New England BioLabs). The recombinant fragment of F2 domain spontaneously generated by proteolytic degradation in bacteria was purified by affinity chromatography on NTi-Agarose followed by FPLC on Superdex 200 column. The correct molecular mass of the purified, recombinant fragment (26 300) was estimated by mass spectrometry. N-terminal sequencing (a.a. res. MEE) identified proteolytic cleavage site in F2 domain. The proper conformation of the recombinant F2 domain fragment was confirmed by circular dichroism analysis. This fragment showed functional activity and could specifically bind to human erythrocytes. The binding was abolished by trypsin and neuraminidase rbc treatment. The presented studies for the first time describe expression and purification of functional recombinant fragment of *P. falciparum* EBA-140 binding region and confirmed a prevalence of F2 domain in human erythrocyte binding.

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P15-14
Self-cleaving ribozyme at the mRNA 5′-end of the *Trypanosoma cruzi* L1Tc retrotransposon

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Retrotransposons are DNA elements able to mobilize via an RNA intermediate to a new genomic position modifying gene expression and genome architecture. Among them, Long Interspersed Nuclear Elements (LINEs) encode their own mobilization machinery and transpose by a mechanism termed Target Primed Reverse Transcription (TPRT). After transcription of these elements, the generated RNAs are used both for translation and as template to generate by reverse transcription a new copy that will be mobilized. The element's transcripts may be generated by internal promoters or to be co-transcribed by a host promoter located upstream the element. The *Drosophila* R2 retrotransposon RNA is released from its co-transcript by an hepatitis deltha virus (HDV)-like ribozyme.

L1Tc is a LINE widely distributed in the genome of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, which contains a functional internal promoter at the element 5′-end (Pr77). We have recently described the presence of an HDV-like ribozyme (L1TcRz) in the 5′-end of the L1Tc RNA, becoming L1Tc the first retrotransposon described with both a promoter and a ribozyme within the same 77 nucleotides. This dual system is also present in other members of the *ingi*-L1Tc retrotransposons clade such as NARTc from *T. cruzi* and *ingi* and RIME from *Trypanosoma brucei*. Trypanosomatids present a singular genetic regulation characterized by polycistronic transcription of large gene clusters as precursors for mature mRNAs. In this context, the existence of the promoter-ribozyme duality in scattered sequences over the genome may play an important regulatory role. Moreover, this dual function reinforces the autonomous character of these elements since they are independent of the host transcription and mRNAs maturation processes.

P15-15
Biochemical evidence of the phylloquinone biosynthesis in extra-erythrocytic stages of *P. falciparum*

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The isoprenoid biosynthesis in *Plasmodium falciparum* by 2C-methyl-D-erythritol-4-phosphate pathway (MEP) is presented as a therapeutic target once it is absent in humans. Several products from this pathway like tocopherol and carotenoids have been characterized although some of them had their biosynthesis described exclusively in photosynthetic organisms. The MEP and shikimate pathways are the precursors of phylloquinone biosynthesis in plants and both have already been described in *P. falciparum*. Using metabolic labeling with [^{3}H]phytyl-PP and [^{3}H]geranylgeranyl-PP, two different methods of RP-HPLC and mass spectrometry analyses we suggested the biosynthesis of phylloquinone might occurs in *P. falciparum*. Moreover, it was demonstrated in *Arabidopsis* sp. a salvage pathway of phytol, a toxic product from degradation of pigments and vitamins that possess a phytyl side chain, resulting in a recycle of this molecule into phylloquinone and tocopherol biosynthesis or in its insertion in the fatty acids of membrane. *P. falciparum* seems to have the salvage pathway like *Arabidopsis* sp, since parasites incorporates [^{3}H]phytol-PP into phylloquinone and tocopherol. Now, we are trying to demonstrate a function as anti-oxidant or electron carrier as described in plants and to find by a bioinformatics approach some putative genes involved in the phylloquinone biosynthesis in order to do a rational study of the enzymes and their inhibitors. Supported by FAPESP and CNPq.

P15-16
Interactions between *Streptococcus pneumoniae* and the defense collagens of the innate immune system

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Streptococcus pneumoniae is a major human pathogen, causing pneumonia, meningitis and septicemia. To insure its survival and dissemination, the pneumococcus deploys an array of virulence factors promoting invasion and evasion from the immune system. Those virulence factors are for most of them proteins associated with the bacterial surface. Besides membrane-associated proteins, LPxTG proteins, choline binding and moonlighting proteins are associated with the pneumococcal surface.

Moonlighting proteins are conserved cytoplasmic metabolic enzymes or molecular chaperones localized at the cell surface and exhibiting additional activities. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been found at the surface of a large range of organisms, bacteria, fungi, parasites, eukaryotic cells and display diverse roles in the virulence process of pathogenic organisms. The pneumococcal surface GAPDH acts as a virulence factor by binding to host plasminogen/plasmin, which facilitates the bacterial invasion through extracellular matrix and endothelial and epithelial cell barriers.

GAPDH is present at the surface of human apoptotic cells and appears to be a ligand for C1q, a defense collagen involved in both apoptotic cells and *S. pneumoniae* clearance. This observation led us to test the recognition of pneumococcal GAPDH by C1q, which indeed occurs both *in vitro* and *in vivo*. We described and characterized this interaction at the molecular level. Our work aims to decipher the molecular mechanisms of crossed innate immune response between pathogens and altered self. The main question is to define if the pneumococcus exploits C1q recognition to evade from the immune system by mimicking the tolerogenic recognition of apoptotic cells.

P15-17
1-deoxy-D-xylulose-5-phosphate synthase from *Plasmodium vivax*

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Malaria is caused in man by an infection by one of five species of protozoans from the genus Plasmodium, *P. falicparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Most of the effort to control and treat malaria has been targeted against *P. falciparum* malaria. However, *P. vivax* malaria is debilitating and can be fatal.

Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are the precursors to a large family of isoprenoid natural products, crucial to the survival of Plasmodium species. For many years, it was thought that IPP and DMAPP were derived solely from mevalonate. A mevalonate independent

or non-mevalonate pathway (NMVA) for the biosynthesis of IPP and DMAPP has been discovered recently; a pathway that supplies isoprenoid precursors in Plasmodium and other parasites. The NVMA pathway of isoprenoid biosynthesis is not found in humans and represents an exciting opportunity for the development novel drugs to treat malaria.

The NMVA pathway consists of eight reactions with the net conversion of pyruvate and D-glyceraldehyde-3-phosphate to CO2 and IPP and DMAPP. The first and rate-determining step of pathway is the condensation of pyruvate and D-glyceraldehyde 3-phosphate to yield 1-deoxy-D-xylulose 5-phosphate and CO2. The enzyme catalyzing this reaction, 1-deoxy-D-xylulose-5-phosphate synthase (DXS), is an attractive drug target.

DXS and the other enzymes of the NMVA are expressed in the nucleus and are translocated to the apicoplast. The Plasmodium species employ a bipartite sequence for translocation of the NMVA pathway enzymes to the apicoplast. This bipartite sequence consists of a signaling and a transit peptide. We have used a step wise deletion approach to produce a soluble and catalytically active form *P. vivax* DXS. This catalytic unit of *P. vivax* DXS, which lacks the signaling and transit peptide, has been successfully overexpressed in *E. coli*. *P. vivax* DXS has been characterized and compared to DXS enzymes from other organisms. Steady-state kinetic parameters and biochemical features are consistent with the DXS reported from other species.

P15r-18
Deletion in promoter region from Toll-like receptor 2 showed no association with susceptibility to human papillomavirus infection

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Introduction: Human Papillomavirus (HPV) is a DNA virus, which has been detected in approximately 99% of cervical carcinoma cases. The immune system is responsible for host defense against possible microorganism invasion. Toll-like receptors (TLRs) are the first line components of the innate immune response. TLR2 is a membrane receptor of cervical tissue but -196 to -174 del polymorphism has been related to the progression of HPV infection and cervical cancer.
Objective: To investigate TLR2 (−196 to -174 del) polymorphism frequency in cervical samples from women infected by HPV.
Materials and Methods: Cervical samples were collected from 234 women attending a Family Health Unit in the city of Olinda, Brazil. DNA was extracted using a Promega kit. HPV infection was investigated by consensus PCR (MY09/11 and GP5+/6+) and PapilloCheck® was used for HPV genotyping. Pap test was used for cellular abnormality investigation. TLR2 (−196 to -174 del) polymorphism was investigated by PCR.
Results: In the Pap test, only 2.56% exhibited cellular abnormalities related to HPV, despite HPV-DNA was detected in 23.50% of patients. HPV6 (14.28%) was the most prevalent genotype, followed by HPV16 and HPV11 (both 8.57%). Among 55 HPV positive samples, TLR2 wt/wt was the most prevalent in both low-risk (34.54%) and high-risk (41.81%) HPV genotype groups. On the other hand, TLR2 del/del was found in only one sample, infected by low-risk genotypes (1.44%), and absent from high-risk samples.
Conclusions: These data suggest that TLR2 -196 to -174 del polymorphism cannot be associated with susceptibility to HPV infection in the target population. Further studies could highlight the association between this polymorphism and the appearance of cervical abnormalities.
Keywords: HPV, TLR2 polymorphism

P15-19
Trypanocidal activity of a nanobody-βcyclodextrin complex

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Trypanosoma brucei brucei are extra-cellular parasites that cause African trypanosomiasis, a disease of humans and livestock in Africa. These parasites use antigenic variation of their surface antigens as the main defense mechanism against the immune system of their mammalian host. While treatments are available, only a few drugs are registered for use and most cause serious side effects in treated patients or animals and are only suitable for early stage infections.

Nanobodies (Nbs) are functional antibodies formed by heavy chain variable domains derived from *Camelidae* that include their capacity to recognize particular epitopes not recognized by conventional antibodies as well as their improve stability and solubility. In addition they can be used as transport devices of other biological active components due to their high target specificity.

βcyclodextrins (βCD) are cyclic oligosaccharide composed of seven α-D-glucopiranoside units attached by a (1-4) glycosidic bonds. The interior cavity of the molecule is able to host hydrophobic compounds and the exterior wall is enough hydrophilic to be water soluble.

The aim of this work was to generate a Nb-based targeting system for site-specific drug delivery, in order to improve current antiparasite therapies. First, a βCD was labelled with a vinyl sulfone, a functional group that allows coupling to proteins. Next, the functionalized βCD was coupled to a Nb that specifically target trypanosome surface. The generated Nb-βCD complex was able to inhibit cell growth on its own, probably due to the ability of βCD to scavenge essential cholesterol from the cell surface membrane. We have employed the Nb-βCD complex to study the functional role of cholesterol in trypanosome. Data will be presented showing whether Nb-βCD complex affects cell cycle, lipid rafts composition, structure and fluidity of the plasma membrane in *T. brucei*.

P15r-20
Molecular approaches to the studies of virulence and apicoplast biology of *Plasmodium vivax*, a neglected human malaria parasite

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The recent call for malaria eradication has put a new emphasis in *Plasmodium vivax*, the most widely distributed human malaria parasite with a population at risk of 2596 billion people and

responsible each year for 100–300 million clinical cases including severe disease and death. With the completion of the genome sequence of *P. vivax*, the need to develop enabling technologies for functional gene discovery is a priority. In the absence of an effective continuous *in vitro* culture system for blood stages and reverse genetics, we are thus exploring the use of heterologous transfection for functional gene discovery. Our group has concentrated efforts in trying to understand the function of variant virulence proteins. We hypothesized that virulence proteins have different subcellular localizations and functions. We have generated 3D7 *P. falciparum* transgenic lines expressing VIR proteins [vir17 like (Subfamily A), vir14 (Subfamily C) and putative-vir10 (Subfamily D)] with different motifs, protein domains and secondary structures. Our results showed that virulence proteins have different subcellular localizations. Significantly, one of these proteins was exposed at the membrane of infected red blood cells and mediated cytoadherence to the ICAM-1 receptor. Adhesion of the transgenic line 3D7 vir14-3HA to other human cell lines will be presented. To further demonstrate the specificity of these findings, we generated a transgenic line of *P. falciparum* expressing a *P. vivax* RNA Binding Protein putatively targeted to the apicoplast. This is an essential organelle of malaria parasites of plastid origin and whose unique metabolic pathways and biology are the targets for therapy. Subcellular location assays, transcription and phenotypical analysis will also be presented.

P15r-21
Antigenicity of *Leishmania infantum* translation initiation factors in natural and experimental visceral leishmaniasis

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Summary: Canine visceral leishmaniasis is a disease caused by the infection of dogs with *Leishmania infantum* protozoan parasites. This form of leishmaniasis is endemic in many Mediterranean countries including Spain. Diagnosis of visceral leishmaniasis cases depends on the discovery of parasites forms in the tissues from infected individuals or in the detection of circulating antibodies against parasite antigenic proteins. Characterization of parasite antigenic proteins will help to the development of second generation serodiagnostic tests. We have evaluated the antigenicity of different translation initiation factors using the sara from hamsters experimentally infected with *L. infantum* and recombinant version of the parasite proteins. The ELISA method was employed to characterize the most antigenic proteins, and to analyse their diagnostic properties. Studies have been extended to dogs affected by visceral leishmaniasis due to *L. infantum* infection. Comparative assays were: (i) between hamsters experimentally infected and naturally infected dogs, (ii) between results obtained in the ELISA made with parasite total protein extracts and recombinant translation initiation factors assayed individually and (iii) between results obtained in the ELISA made with parasite total protein extracts and mixtures of the most reactive recombinant translation initiation factors. The possibility of developing a diagnostic test for canine visceral leishmaniasis based on this protein family is discussed.

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P15r-22
Transport and metabolism of L-tryptophan and L-kynurenine in *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of Chagas's disease, a chronic, debilitating and incurable zoonosis that it is found mainly in Latin America, covering 21 countries and killing more than 10 000 people annually. This organism spends part of its lifecycle in glucose-poor environments so amino acids are important sources of energy for its development. Tryptophan (trp) catabolism has risen in recent years as a significant immunomodulatory mechanism in several infectious diseases. Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme that catalyzes the transformation of trp into kynurenine (kyn) leading to kynurenine pathway that generates some relevant intermediate metabolites (globally known as kynurenines). These kynurenines play an important immunological role in limiting tissue damage caused by a strong pro-inflammatory response against pathogens. In this work, we explore the potential role of the parasite itself in the modulation of trp and kyn serum levels in the host. We present the first biochemical description of two active trp transport systems in epimastigotes of *T. cruzi*. Uptake of this amino acid occurs by a high affinity low capacity system (low) and a low affinity and high capacity system (high). Both systems have relatively low substrate specificity as they are inhibited by several amino acids in a differential way. Interesting, from an immunological point of view, is the fact that kyn is able to inhibit both systems and it is rapidly metabolized by the parasite. We also provide evidence indicating that kyn may be exchanged by trp, suggesting that *T. cruzi* could modulate the tryptophan / kynurenine ratio in the local host environments.

P15-23
Structural insights on the molecular basis of the non-cooperative behavior of glyceraldehyde-3-phosphate dehydrogenase from *Trypanosoma cruzi*

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Glyceraldehyde-3-phosphate dehydrogenase from *T. cruzi* is a homotetramer of four identically subunits that requires the cofactor NAD^+ for activity. The enzyme catalyzes the reversible oxidative phosphorylation of D-glyceraldehyde 3-phosphate (GAP) into D-glycerate 1,3-bisphosphate (1,3-diPG) in the presence of NAD^+ and inorganic phosphate (Pi). The enzyme is a validated target for the survival of the parasite, but still presents properties that remain to be explained at the molecular level, including the observed non-cooperativity upon NAD^+ binding, differently from other homologous enzymes, including the human ortholog. With the aim of shedding light on the structural basis underlying this behavior, X-ray crystallography studies were conducted. Four independent TcGAPDH structures were obtained in the presence of an increasing number of NAD^+ bound to the tetrameric particle catalytic sites, namely the *apo* form, 1NAD, 2NAD and *holo*). The comparison amongst the structures revealed that the enzyme undergone an approximate rigid-body rotation upon NAD^+ binding. Similar results were observed for partially saturating structures 1NAD and 2NAD. In the case of

1NAD structure, the enzyme can be described as a tetramer made up of three subunits in the apo conformation and the fourth subunit, containing the NAD^+, adopting the holo conformation, whilst the 2NAD structure, present two subunits in the apo conformation and the other two, where NAD^+ molecule is bound, in the holo conformation. Therefore, the structural data are in good agreement with the kinetics results. Structural analysis and site direct mutagenesis studies showed that the intersubunit residue Thr54 is important for the non-cooperative behavior. The information collected in this work contribute to better understand the molecular basis of the cooperative behavior in GAPDHs, including the identification of a non-competitive inhibitors that binds at the oligomeric interface.

P15-24
Targeting drugs into *Leishmania mitochondrion*. Inhibition of succinate dehydrogenase (complex II) as key activity for the leishmanicidal mechanism of diphenyl and bisphosphonium salts

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Among the protozoal infections Leishmaniasis ranks second only to malaria. Chemotherapy is the only way to fight the disease; its efficacy is threatened by the scarce number of drugs and rampant resistance. Thus, development of new drugs is an urgent need. Among the different alternatives to explore, mitochondrial respiration is essential for parasite viability, thus, an excellent target for new leishmanicidal drugs. Our rationale was to promote mitochondrial import of diphenyl surrogates by incorporation of two phosphonium groups with a positive delocalized charge at 4,4′ position of the scaffold; thus the electrochemical mitochondrial potential will drive the accumulation of these lipophilic dications into the organelle. An initial set of 48 compounds were assayed for inhibition of parasite proliferation and macrophage toxicity. From them, four surrogates with the highest selectivity index (>20) and LC_{50}at low micromolar range were selected. At concentrations causing 70–90% mortality, these compounds induced a fast drop in the intracellular levels of ATP in promastigotes without membrane permeabilization, so inhibition of mitochondrial ATP is the leading cause. This hypothesis was confirmed as the drugs caused (i) Mitochondrial depolarization, assessed by Rhodamine 123 accumulation, (ii) Inhibition of succinate dehydrogenase (Complex II), their main target inside the mitochondrial respiratory chain, by polarographic methods according to the variation of oxygen consumption rate in digitonin-permeabilized parasites. Additionally, the compounds induced mitochondrial swelling in electron microscopy, and apoptosis of the parasites. Altogether, we demonstrated the feasibility of targeting new drug leads into mitochondria by addition of import motives, diphenyl bisphosphonium surrogates resulted as a new and promising scaffold for development of new leishmanicidal drugs.

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P15-25
Deconvolution of eosinophyl cationic protein (ECP) for cell-penetrating and microbicidal activities on *Leishmania*

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Eosinophil cationic protein (ECP), a member of the RNAase A superfamily, plays a crucial role in host defense against bacteria, viruses and parasites. Its lethal mechanism combines enzymatic activity and membrane permeabilization, due to a strong cationic character. The antibacterial activity of ECP has been traced to the N-terminal domain [1]. Here we explore the ability of both ECP and peptides reproducing this N-terminal domain to internalize and eventually kill *Leishmania* parasites. To this end, recombinant ECP and peptides spanning the ECP N-terminus (1-45) were assayed on *L. donovani* promastigotes and on *L. pifanoi* amastigotes for toxicity (EC_{50}), inhibition of proliferation, membrane permeabilization, activity on bioenergetic metabolism and cell internalization by both electron and confocal microscopy.

ECP and three out of 4 N-terminal analogs were leishmanicidal on promastigotes, consistently less on amastigotes. Highest activity was for ECP and ECP (8-45) ($EC_{50} = LD_{50} = 3.4\ \mu M$). Both killed parasites on an all-or-none basis, causing minor plasma membrane disruption yet considerable cytoplasmic disarray. As for internalization, only fluorescently labeled ECP was able to localize inside *Leishmania* parasites, accumulating in the parasitophorus vacuole and reducing parasite load in infected peritoneal macrophages. The consistently higher activity of ECP versus the peptide analogs suggests that, in contrast to the lytic activity on bacteria, on *Leishmania* ECP appears to act through a yet unveiled intracellular target. Further, the effect on intracellular parasites reveals ECP as a useful candidate for *Leishmania* chemotherapy as well as for ferrying payloads into the parasite.

References

[1] Torrent et al., J Med Chem 54(14):5237-44.(2011) Projects: RICET RD 06/0021/0006, ISCIII PI09-01928 to LR, HEALTH-2007-223414 to LR and DA, SAF2011-24899 to DA,BFU2009-09371 to EB.

P15-26
Proline-based γ-peptides as novel cell penetrating peptides (CPPs) in *Leishmania*

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Cell penetrating peptides (CPPs) translocate across biological membranes through their interaction with phospholipids, delivering a wide variety of molecules conjugated to them. Two of the factors involved in CPP translocation -membrane composition and endocytosis- show strong peculiarities in Trypanosomatids compared to mammalian cells, such a sturdy plasma membrane, endowed with a rich anionic glycocalix and strong proteolytic activity, and endocytosis confined exclusively into the flagellar pocket, a specialized area of the plasma membrane. This poses a challenge for CPPs tested on mammalian cells.

We have assayed on *Leishmania donovani* promastigotes and *L. pifanoi* amastigotes a γ-peptide library based on a (2S, 4S)-4-amino-L-proline backbone with its a-amino group functionalized with a variety of side chains differing in charge, hydrophobicity and class of functional group.

None of the peptides assayed turned out to be toxic up to 25 μM. We selected three with a better internalization than Tat - a standard CPP- as evaluated by flow cytometry and confocal microscopy. Two of them were homogenously substituted with the alkyl or acyl side chain of arginine whereas the third was with a quaternary amine. Such combination of either delocalized or low density positive charge together with the hydrophobic skeleton of the peptide, is essential for peptide translocation. Peptides enter *Leishmania* through endocytosis, as they co-localize with Rhodamin B-dextran.

Their good internalization rate as well as stability towards proteinase degradation, make γ-peptides promising candidates to ferry cargo molecules into *Leishmania*, a parasite dwelling inside highly proteolytic environments.

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P15r-27
Characterization of actin nucleating Arp2/3 complex in *Trypanosoma brucei*

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African trypanosomiasis is a widespread tropical disease transmitted to their vertebrate host through the bite of the tsetset fly. *Trypanosoma brucei rhodesiense* and *T. b. gambiense* cause human sleeping sickness, and *T. b. brucei* causes nagana in livestock. The parasites multiply in the blood and escape the immune response of the infected host by antigenic variation.The disease is fatal if not treated, however very few efficient drugs are currently available. The aim now is to translate basic research on these parasites into effective therapeutic action. In terms of parasite vulnerability, the endocytic machinery is and obvious target for such action because bloodstream forms are dependent on receptor-mediated uptake of host-derived factors for growth and have high rates of endocytosis. Actin is essential in bloodstream forms of *Trypanosoma brucei* and is required for the formation of coated vesicles. Inhibition of actin polymerization indicated that the assembly of filamentous actin is the key step.

The aim of this work has been the characterization of the universal actin nucleating Arp2/3 complex in *T. brucei*. In bloodstream forms Arp2 is located in a region between the nucleus and the kinetoplast corresponding to the endocytic pathway. Interestingly, Arp2 is also present in a ring structure near the nucleus at the posterior end of the cell body. Protein depletion by RNAi resulted in cell grow inhibition, cell cycle arrest and endocytosis inhibition leading to gross enlargement of the fagellar pocket. The same results were observed with latrunculin b, an inhibitor of F-actin polymerization. These results validate the actin cytoskeleton as a therapeutic target in African trypanosomes.

P15r-28
In vitro culture of *Cryptosporidium* spp. oocysts in the novel coe cell lines derived from lamb umbilical cord

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The aim of this work was obtaining and characterizing cell cultures derived from lamb umbilical cord apparently healthy and showing its industrial applicability for productive growth of some pathogenic intra cellular parasites, e.g. Cryptosporidium spp.

Protozoan parasites of the genus Cryptosporidium cause diarrhea in many vertebrate animals, including humans. Its medical relevance as a dangerous opportunistic parasite and widespread water contaminant has been fully recognized as an emerging zoonotic Apicomplexa protozoa. The *in vitro* systems for *Cryptosporidium* spp. propagation has been a major obstacle for researching this parasite.

To obtain COE cell line, primary culture was prepared in Eagle's Minimum Essential Medium, supplemented with 10% fetal bovine serum, antibiotics and HEPES. Cell morphology was characterized as epithelial in the 35 subcultures. Besides, aneuploid and hipodiploid karyotype with modal class of 34 chromosomes was observed; Cell line is free of adventitious agents, *Mycoplasm* spp., Prions and Pestivirus.

We cultivated *Cryptosporidium* spp from samples of feces and animal's intestine (calves, goat, and sheep) with diarrhea and inmunochromatography and histopathological lesions documented.

Cryptosporidium spp. in COE cells lines achieved great multiplication of parasites and complete development from sporozoite to sporulated oocyst. Forms in the cytoplasm of the cells and extracytoplasmic location (zygotes and spores with eight sporozoites) were exhibited. Percentages of cell parasitism in intracellular and extracellular stages were increased about 70–80% respecting to control cultures. Morphological data, specific immunofluorescence and real-time PCR 18S ribosomal gene were used to confirm these results.

This cell line is a biological system for the recovery of oocysts on a media-large *in vitro* scale.

P15-29
Regulation of malarial protein kinases PfPKB, PfCDPK1 and host cell invasion by calcium signaling

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Calcium is crucial for the development of malaria parasite *Plasmodium falciparum*. One of the major roles of calcium is to regulate the invasion of the host RBCs. Phospholipase C (PLC) mediated calcium release may be critical for invasion. The inhibition of PfPLC in the parasite blocks the activation of protein kinases PfPKB and PfCDPK1 suggesting that these kinases may be effectors of calcium signaling in the parasite. Gene disruption studies suggest that PfPKB and PfCDPK1 are essential for parasite growth and their inhibition by pharmacological inhibitors prevents invasion. Studies directed at understanding how calcium regulates PfCDPK1 suggested: (i) inter-domain interactions are essential for stabilizing the inactive and the active form of the kinase; (ii) autophosphorylation of various domains is essential kinase activation; (iii) the N-terminal extension of the enzyme is

regulatory and contains an ATP-binding site. PfGAP45, an important component of the glideosome complex, which anchors the actomyosin motor in the merozoite, was identified as a target for both PfCDPK1 and PfPKB. The regulation of PfGAP45 by calcium mediated signaling and by these was studied. Collectively, these studies provide novel insights on calcium signaling pathways involved in the invasion of host RBCs by malaria parasite and help understand molecular mechanisms mechanism involved in the regulation of key components of these pathways.

P16 – Neuroscience

P16-1
Impact of DPP IV/CD26 deficiency on neuropeptide Y among the gut-brain axis in experimental colitis

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Inflammatory bowel diseases (IBD) are a group of chronic inflammatory conditions of the gastrointestinal tract with unclear etiology. A bidirectional connection between central and enteric nervous systems and the role of peptidases in maintaining the homeostasis in the gut via bioactive substrates has been proposed. Dipeptidyl-peptidase IV (DPP IV/CD26), a multifunctional glycoprotein found in soluble and membrane-bound form, whose role in immune-mediated diseases was described. The involvement of neuropeptide Y (NPY), a substrate of DPP IV/CD26, in experimental colitis has been indicated. Our hypothesis was that DPP IV/CD26 plays an important role in IBD by influencing the circulating and tissue levels of NPY in an experimental model of IBD. In order to evaluate the impact of DPP IV/CD26 on NPY levels among the gut-brain axis, a Crohn-like model of colitis has been induced in CD26 deficient and wild type (C57BL/6) mice. Decreased DPP IV/CD26 activity was found in serum, colon and brain in wild type mice with colitis, while CD26 expression was increased in colon. Inflammatory events in the colon lead to an increase in serum and colon NPY concentrations in both mice strains. Serum NPY changes were more emphasized in CD26 deficient mice, while NPY colon concentration was higher in wild type. Furthermore, we determined that colitis induces an increase in brain NPY concentration in the acute phase in wild type mice and, adversely, a decrease in CD26 deficient mice. Results of this study have shown that changes occurring during inflammatory processes in colon reflected on investigated parameters in brain. Our results indicate the importance of the gut-brain axis in the pathogenesis of IBD, as well as an important impact of DPP IV/CD26 on NPY during experimental colitis.

P16-2
Lowering cholesterol and neuroprotection

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In our earlier work, lowering cholesterol increased the extracellular glutamate level in rat brain synaptosomes that was a result of the lack of transporter-mediated glutamate uptake. In stroke, cerebral hypoxia/ischemia, traumatic brain injury, the development of neurotoxocity is provoked by enhanced extracellular glutamate, which is released from nerve cells mainly by glutamate transporter reversal - a distinctive feature of these pathological states. Under these pathological conditions, cholesterol-lowering drugs statins have neuroprotective features. The aim of this study was to assess transporter-mediated release of glutamate from cholesterol-deficient synaptosomes. Cholesterol acceptor methyl-*b*-cyclodextrin reduced the cholesterol content in synaptosomes by one quarter. Transporter-mediated glutamate release from synaptosomes: (i) stimulated by depolarization of the plasma membrane; (ii) by means of heteroexchange with competitive transportable inhibitor of glutamate transporters DL-threo-b-hydroxyaspartate; (iii) in low [Na^+] medium; and (iv) during dissipation of the proton gradient of synaptic vesicles by the protonophore FCCP; was decreased under conditions of cholesterol deficiency by appx. 24, 28, 40, 17%, respectively. A decrease in the level of membrane cholesterol attenuated transporter-mediated glutamate release from nerve terminals. So, lowering cholesterol may be used for neuroprotection just after stroke, cerebral hypoxia/ischemia, traumatic brain injury, which are associated with an increase in glutamate uptake reversal. This data can explain neuroprotective effects of statins in these pathological states and provide one of the mechanisms of their neuroprotective action. However, beside these disorders lowering cholesterol may cause harmful consequences decreasing glutamate uptake by nerve terminals.

P16-3
Network of brain protein level changes in glutaminase-deficient mice

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Capsule: Background: Brain glutaminase is a multifunctional enzyme. Glutaminase knock out mice die in the first postnatal day; heterozygous mice show a subtle behavioral phenotype.
Results: Using a gel-based proteomics approach in glutaminase deficient mice, two networks of glutaminase-dependent brain proteins were identified.
Conclusion: Glutaminase deficiency affected networks involved in cellular assembly, cycling, organization and signaling.
Significance: In late fetal life, glutaminase plays important roles in broad networks involved in brain cell maturation.
Abstract: Glutaminase (gene GLS1) is a multifunctional enzyme involved in energy metabolism, ammonia trafficking and regeneration of neurotransmitter glutamate. GLS1 knockout-mice are born smaller, have impaired glutamatergic synaptic transmission in brain stem respiratory nuclei and die shortly after birth due to a paradoxical hypercapneic response; GLS1 heterozygous mice (GLS1+/Δ) are remarkably normal, with a subtle phenotype of schizophrenia resilience. To address the protein basis for the phenotypes of the glutaminase-deficient mice, brain proteins from late gestation wild type, GLS1+/Δ and GLS1Δ/Δ male mice were extracted, run on two-dimensional gel electrophoresis, with subsequent identification by mass spectrometry using nano-LC-ESI-MS/MS. Protein spots that showed differential genotypic variation were quantified by immunoblotting. GLS1 protein showed the expected genotypic reductions. Differentially expressed proteins unambiguously identified by MS/MS included neurocalcin delta, retinol binding protein-1, reticulocalbin-3, cytoskeleton proteins fascin and tropomyosin alpha-4-chain, dihydropyrimidinase-related protein-5, apolipoprotein IV and proteins from protein metabolism proteasome subunits alpha type 2, type 7, heterogeneous nuclear ribonucleoprotein C1/C2 and H, voltage-gated anion-selective channel protein 1 and 2, ATP synthase subunit ß and transitional endoplasmic reticulum ATPase. Interaction networks determined by Ingenuity Pathway Analysis revealed a link between glutaminase and calcium, Akt and retinol signaling, cytoskeletal elements, ATPases, ion channels, protein synthesis and the proteasome system, intermediary, nucleic acid and lipid metabolism, huntingtin, guidance cues, transforming growth factor beta-1 and hepatocyte nuclear factor 4-alpha. The

two principal networks identified involve (i) cellular assembly and organization and (ii) cell signaling and cell cycle suggesting that GLS is crucial for neuronal maturation.

P16-4
Structural and functional interactions of Alpha-Synuclein with mithocondia

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Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the Substantia Nigra of the midbrain. Alpha-Synuclein (AS) is a 15 kDa protein expressed in the central nervous system and is the major component of the protein aggregates in PD. It was proposed that in early steps of AS aggregation, toxic oligomeric species are formed that may lead to mitochondrial dysfunction, fragmentation and apoptosis.The aim of this work is to characterize the interaction of AS with mitochondrial membranes in intact organelles and the effect of AS on mitochondrial function. Mitochondria were isolated from SH-SY5Y cells and incubated with fluorescent labeled AS (0, 1 and 10 μM). The outer membrane was revealed by immunofluorescence of TOM20. A combined study using confocal and widefield microscopy with deconvolution and 3D reconstruction revealed a different localization of AS dependent on the concentration. At 1 μM AS the protein colocalized with TOM20 thus showing an outer membrane distribution, which was clustered according to high resolution STED microscopy. When mitochondria were incubated with 10 μM AS, there was a preferential localization of the protein in the interior of the organelle. Mitochondrial activity was affected by the incubation with AS. When mitochondria were stained with mitotracker, a dye that stains only active mitochondria, we found a reduction of the signal in the organelles incubated with 10 μM AS. Activity of mitochondrial complex IV was also inhibited, while complex I-III and II-III appears to be unaffected. This results reveals for the first time a concentration dependent AS internalization in isolated mitochondria that correlates with the inhibition of the mitochondrial activity.

P16-5
Oxidized cell-free DNA as an emergency signal for the nervous system cells

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Cell-free DNA (cfDNA) can accumulate in body fluids with the organism's compensatory mechanisms failure during pathological processes. Neurodegenerative diseases are characterized by the appearance of oxidized deoxyguanosine (dGox) in the cerebrospinal fluid (CSF). We proposed this event is due to accumulation of oxidized cfDNA in CSF. The purpose of this study was to determine the effect of oxidized cfDNA on nerve cells viability and assume its function in the nervous system.

Methods: We prepared granule neurons culture (GNC) from cerebella of 6- to 8-day postnatal Wistar rats. Genomic DNA (gDNA) from rat brain was isolated and DNA samples with different lengths of the fragments and the degree of oxidative modification (treatment with methylene blue and H_2O_2) were prepared. The content of oxidized deoxyguanosine in DNA samples was evaluated by HPLC-mass spectrometry. The effect of model gDNA and gDNAox samples (1–50 ng/ml) on heterogeneous culture of cerebellum cells in normal and glutamate excitotoxicity conditions was investigated.

Results: Native gDNA caused DNA secretion from neurons the most pronounced 3 hour after the application. In the case of gDNAox the reaction was delayed – the peak of secretion was observed after 4 hour. The comet-assay showed double-stranded breaks formation in neurons DNA after its exposure to both gDNA and gDNAox. In contrast to the native gDNA neuroprotective effect observed in glutamate excitotoxicity increasing from 5 to 50 ng/ml gDNAox even in low concentrations reduced neurons survival when applied 2 hour before or 0.5 hour after glutamate treatment. Normally gDNAox induced the suppression of reactive oxygen species production in neurons and astrocytes activation. These data suggest that cfDNA is an emergency signal for the nervous system cells.

P16-6
Synthetic neuropeptide regulation of GABA(A) receptor-agonist interactions on rat brain cells plasmatic membranes

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Peptide complexes in living organism well known as an universal regulators of practically all of physiological functions. Neuropeptides and their modified analogues take especial pharmacological attention for neurodegenerative disorders. The processes underlying biological activity of original analogues of native neuropeptides and their short derivatives keeps unclear. We tried to mark out the key process of peptide action molecular mechanism – specific interactions on brain cells plasmatic membranes and peptide ability to modulate endogenous ligand-receptor interactions. Our recent investigations shows that some synthetic neuropeptides analogues is able to specific binding to P2 brain cells membranes and have an influence on specific ligand-receptor binding of well-known signal molecules to their receptors. We shows possibility of selected regulatory peptides to change affinity of [^{3H}]GABA binding to GABA(A) receptors. Also we tested different sites of GABA(A) receptors in regulatory peptides presence by using specific ligands (such as neurosteroid, benzodiazepine, ethanol and e.t.c.). All data observed let us to seem regulatory peptides as effective allosteric regulators of GABA(A) receptors function. This work let us to bring closer the understanding of fundamental basis of molecular mechanism underlying the regulatory peptides biological action.

P16-7
Cholinesterase inhibition and destabilization of beta-amyloid fibriles with novel N′-2-(4-Benzylpiperidin-/piperazin-1-yl) acylhydrazone derivatives

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A novel series of N′-2-(4-Benzylpiperidin-/piperazin-1-yl) acylhydrazone derivatives have been designed, synthesized and evaluated as anti-Alzheimer's disease ligands. The enzyme inhibition data indicated that newly synthesized compounds inhibit both acetylcholinesterase (ACh) and Butyrylcholinesterase (BuChE).

Beta-amyloid (AB) aggregation results showed that all compounds exhibited highest AB-fibril aggregation/inhibition activity with a similar potential as the reference compound rifampicine showing their potential as dual inhibitors for cholinesterases and AB aggregation. Inhibition mechanism of the most active compounds were investigated also by molecular docking experiments.

P16-8
New generation of penetrating cations as potential agents to treat ischemic stroke

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Several ischemia-induced neurological conditions including stroke involve pathologies with high level of oxidative stress. An antioxidative strategy is needed with specific attention to mitochondria both as a source of, and a target for reactive oxygen species accompanying oxidative stress associated with ischemia. Thus, mitochondria-targeted compounds with antioxidative and/or 'mild' uncoupling properties potentially may be beneficial in the treatment of the brain ischemia and its consequences.

The goal of our study is to evaluate neuroprotective potential of the mitochondria-targeted chimeric compounds characterized by antioxidative and 'mild' uncoupling properties implying a model of the middle cerebral artery occlusion (MCAO).

We tested mitochondria-targeted chimeric compounds consisting of either a rhodamine 19 or B and phosphonium moiety linked to a plastoquinone molecule named SkQR1 or SkQRB and SkQ1 correspondingly. Among all tested compounds the highest antiischemic efficiency was displayed by SkQR1. We found that a single i/p injection of SkQR1 at dose 0.5, 1, 2 mM/kg before or after MCAO significantly diminishes infarct volume and neurological deficit of ischemic animals in a dose-dependent manner. SkQRB and SkQ1 did not decrease brain injury but contributed to neurological regression. An analog of SkQR1 not containing plastoquinone (C12R19) but carrying protonophorous ability significantly decreased neurological deficit of ischemic animals but not the infarct volume. 'Classical' uncoupler, 2,4-dinitrophenol did the same. We also revealed that SkQR1 activates signaling pathways involved in ischemic tolerance induction. We conclude that beneficial neuroprotective effect of rhodamine 19 derivative of mitochondria-targeted compound SkQR1 may be at least partially explained by a direct antioxidative and uncoupling effect of the drug. In addition, we cannot exclude some other mechanisms of SkQR1 action, in particular, through a mechanism of ischemic tolerance induction.

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P16-9
Endocytic adaptor protein intersectin 1 (ITSN1) interacts with cytoskeletal protein STOP in neurons

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Intersectin 1 (ITSN1) is an adaptor protein that is involved in clathrin-mediated endocytosis. There is increasing evidence that this protein is associated with development of some neuropathologies, namely Down syndrome and Alzheimer's disease. ITSN1 is important for neuronal functioning as evidenced by existence of its neuron-specific isoforms and their high level of expression. However, despite the data of ITSN1 involvement in synaptic vesicle recycling and dendritic spine development its role in functioning of neurons is still unclear.

In order to determine the composition of neuronal ITSN1-related protein complexes we performed search for novel ITSN1 neuron-specific interacting proteins. For this purpose recombinant GST-fused SH3 domains of ITSN1 were expressed in bacteria and used as bait in *in vitro* binding assay with mouse brain total protein lysate. For SH3A domain we observed an unknown band of 125 kDa which was identified by MALDI-TOF mass spectrometry as STOP (stable tubule-only polypeptide). This protein is a main factor that determines Ca2 + /calmodulin-regulated microtubule cold and drug stability and also takes part in generation of synaptic plasticity. Since glial cells contain STOP isoforms with smaller molecular weight, we concluded that STOP is neuron-specific binding partner of ITSN1. Results on mass spectrometry were confirmed by GST-pull-down and co-immunoprecipitation of ITSN1-STOP complexes using anti-STOP and anti-ITSN1 antibodies. Finally, with both fluorescent antibodies and recombinant FP-tagged proteins we determined subcellular localization of these proteins in rat primary hippocampal neurons. Additional experiments will be performed to elucidate functional meaning of this interaction.

P16-10
Adenosine A2AR blockade prevents retinal microglial activation

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Adenosine is a neuromodulator in central nervous system (CNS) that acts on four receptors: A_1, A_{2A}, A_{2B} and A_3.The majority of cells involved in the pro-inflammatory responses express adenosine receptors. Microglial cells are the resident innate immune cells in the CNS. In response to pro-inflammatory stimuli, microglial cells become activated, releasing inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) and nitric oxide (NO). Increasing evidence has demonstrated that A2AR blockade may prevent neurodegeneration in the brain by modulating the release of noxious factors by activated microglial cells. However, currently, relatively little is known about microglial cells homeostasis in the retina. Since adenosine receptors (AR) are expressed in the retina, the aim of this work was to evaluate the effect of A2AR blockade in the control of retinal microglia reactivity induced by an inflammatory stimulus.

Purified retinal microglial cell cultures were pretreated with CGS21680 (30 nM, A2AR agonist) or SCH58261 (50 nM, A2AR antagonist), and challenged with lipopolysaccharide (LPS, 1 μg/ml) to mimic an inflammatory stimulus. Cultured retinal microglial cells express A2AR and treatment with LPS increased A2AR immunoreactivity. Activation of A2AR potentiated NO production induced by LPS, while A2AR blockade prevented NO increase. Moreover, A2AR blockade inhibited the LPS-induced increase in TNF-α expression and decreased the percentage of cells phagocyting beads, suggesting that blockade of A2AR can prevent LPS-induced phagocytic activity. Preliminary results using cultured retinal explants also indicate that A2AR blockade inhibited the increase in iNOS immunoreactivity triggered by LPS.

Our results indicate that modulation of A2AR activity may control retinal microglial cell reactivity. Taking into account the

role of microglial cells in neuroinflammation, these data open the possibility for the use of A2AR antagonists in retinal degenerative diseases involving inflammation.
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P16-11
Effects of extremely low-frequency electromagnetic fields on neurotrophic factor levels and behavior changes in neurotoxicity induced by 3-nitropropionic acid: neuroprotective effect

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Data indicate that the neuroprotective effect of extremely-low-frequency electromagnetic fields (ELFEF) may be due to the action on neurotrophic factors levels and cell survival, leading to an improvement in behavior. This study investigated the neuroprotective effects of ELFEF in a rat model of 3-nitropropionic acid (3NP)-induced Huntington's disease. Behavior patterns were evaluated and changes in neurotrophic factor levels were monitored in Wistar rats. Rats were given 3NP over four consecutive days (20 mg/kg BW), while ELFEF (60 Hz and 0.7 mT) was applied over 21 days, starting after the last injection of 3NP. Rats treated with exhibited significantly different behavior, and displayed significant differences in neurotrophic factor levels with respect neuronal controls. ELFEF improved neurological scores and enhanced neurotrophic factor levels in 3NP-treated rats.

P16-12
Induced adipogenic stems cells are valuable for therapy of human diseases: stroke and neurodegenerative diseases

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Action of inducers of neuronal differentiation (β-mercaptoethanol, GNDF, BDNF, retinoic acid (RA), 5-azacytidine) on cultured human and mouse adipose-derived stem cells (ASCs) and their fate after transplantation into injured and ischemic mouse brains were studied. Three days after, phenotype of induced cells was analyzed using immunocytochemistry and real-time PCR for differential expression of specific genes. ASC cultivation in medium with RA or BDNF in combination with 5-azacytidine for a week increased mRNA and protein levels of nestin, Tub-B and ENO2. Induction effect of BDNF was mediated by receptor TrkB, which mRNA was detected in initial ASCs population. Then induced cells were injected into striatum of Black6 mice. After 9 days, many induced ASCs migrated into surrounding brain parenchyma. Control uninduced cells were arranged locally and did not extend beyond injection site. By 11th day after injection control cells disappeared from brain sections, whereas induced cells remained alive. Data suggest that BDNF with 5-azacytidine not only promotes neural differentiation of ASCs and increases expression of neural genes, but also increases survival of cells after transplantation into brain tissue. Immunohistochemical staining of sections containing transplanted cells showed that induced by descibed method ASCs expressed dablkortin. Transplantation of induced mouse ASCs into mouse brain increased lifespan of cells relative to control uninduced cells and promoted their migration from transplantation site to recipient cerebral parenchyma. Transplantation of induced cells into mouse brain, pre-exposed to endothelin-1, promoted more active cell migration into surrounding ischemic brain tissue.

P16r-13
Vascular niche factor regulate rodent adult carotid body stem cell activity

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Preliminary observations in our lab identified the mammalian carotid body (CB) as a neurogenic niche in the peripheral nervous system with a recognizable physiological function in adult life. This chemoreceptor organ is responsible for the detection of hypoxemia and is able to adapt to a persistent stimulus by increasing the number of neuronal, type I cells. We have previously shown that this neurogenesis process depends on the proliferation and differentiation of a quiescent subpopulation of glia-like stem cells (SCs) which are activated in chronic hypoxia.

We are interested in the mechanisms underlying the activation process in the CB neurogenic niche. We first analyzed the implication of the oxygen tension and whether the SCs are sensitive to the hypoxic stimulus by themselves or need communication from the surrounding vascular cells. Our recent data suggest that hypoxia is not a direct stimulus activating CB stem cell (CBSC) proliferation. On the contrary, endothelin-1 (ET-1), a vascular niche factor released during the hypoxic stimulus, is able to activate neural progenitors.

Regarding the role of ET-1, it has a potent effect on increasing proliferation in CBSCs. We have analyzed ET-1 production in different CB cell types and have determined endothelial cells as the main source for this factor. However, we have evidences that neuronal type I cells could produce ET- 1 in an oxygen dependent manner. Moreover, we have quantified the angiogenesis occurring in the organ in response to hypoxia to highlight the increase in production of ET-1 during the stimulus. This work helps to understand the importance of the niche in SC biology, and specifically the implications of vascular and neuronal cells on directing proliferation and cell fate in CB neural progenitors.

P16-14
Long-term consequences of prenatal exposure to LPS or Poly I:C: effect on behavioural pattern and level of cytokines in adult rats and changes induced by chronic clozapine administration

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Many studies indicate an association between the incidence of environmental insults (such as inflammation) during a vulnerable developmental period of the CNS and an increased risk of the development of schizophrenia in adulthood.

To simulate the effects of maternal infectious processes on the development of the brain and determine their postnatal consequences, this study was aimed at (i) describing the behavioural deviations and cytokine profile alterations in rats systemically prenatally exposed to viral (Poly I:C) and bacterial (LPS) components; (ii) describing the effect of chronic clozapine administration on these alterations.

The pregnant rats (E 18-19) were injected (i.p.) with LPS (0.5 mg/kg) or Poly I:C (5 mg/kg). Behaviour (open field, PPI) was observed both on PD 50 and in terms of the effect of 30 days of clozapine administration (CLZ, 20 mg/kg/day). The delayed effect of the prenatal challenge with Poly I:C on immune system was evaluated as cytokine levels measured by multiplex assay.

Both LPS and Poly I:C-treated rats had unchanged total locomotor activity during the 30-min test, however, both drugs induced the impairment of PPI in PD 50 rats. Dysregulation of cytokine expression was documented in the Poly I:C-treated groups: a lower ratio of IL-10/IFN-gamma on PD 50, with a narrowly non-significant higher level of IL-2, IL-4, IL-6, IL-10, GM.CSF on PD 90 associated with clozapine treatment.

These findings suggest that neonatal immune activation by infectious agents may have long-lasting effects, both on the development of the behavioural phenotype and on the immune system.

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P16-15
Glial P2Y receptors contribute to the regulation of noradrenergic transmission in the rat brain cortex

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Nucleotides released from glial cells, acting on purinergic receptors, modulate, the release of noradrenaline (NE) and astrocytes express transporters that regulate the synaptic concentration of NE (Gordon et al., 2005, Nat Neurosci 8:1078; Inazu et al., 2003, J Neurochem 84:136). In this study we investigated the subtypes of purinergic receptors that modulate NE transmission, in rat brain cortex. The models used consisted of brain slices to evaluate the effects on NE release evoked by electrical field stimulation (EFS) and glial cultures to investigate the effect on the NE-uptake by glial cells. Results are expressed as % change from controls as mean ± SEM from n experiments. Neither the A_{2A} selective agonist CGS21680 nor the A_3 selective agonist Cl-IB-MECA changed NE release, but the selective A_1 receptor agonist CPA (0.001–10 μM) and several P2 receptor agonists (0.01–1 mM) inhibited NE release up to 58%, with the order of potency: CPA > ADP ≥ ATP > ADPβS > UTP > UDP. The effect of CPA was abolished and that of adenine nucleotides and UDP were attenuated by the selective A_1 receptor antagonist DPCPX (0.1 μM). The effect of ADP (0.3 mM; 48 ± 2%, n = 10) was also attenuated by adenosine deaminase (ADA 2 U/ml; 30 ± 5%, n = 5) and by the selective $P2Y_1$ antagonist MRS2500 (1 μM; 23 ± 4%; n = 6) being abolished by MRS2500 plus DPCPX. ADPβS (0.3 mM) a similar effect to ADP (0.3 mM), except that it was not changed by ADA. The effects of UTP (1 mM; 32 ± 3%, n = 6) and UDP (1 mM; 32 ± 1%, n = 6) were abolished by the selective $P2Y_6$ receptor antagonist MRS2578 (1 μM). In glial cultures, ADPβS, UDP and CGS21680 increased NE-uptake but CPA had not effect. Effect of ADPβS (0.1 mM; 117 ± 15%; n = 5) was attenuated by the $P2Y_{12}$ antagonist AR-C66096 (10 μM; 66 ± 9%, n = 5) and MRS2500 (1 μM; 22 ± 3%; n = 5), that of UDP (1 mM; 96 ± 14%; n = 5) by MRS2578 (1 μM;42 ± 4%; n = 5) and that of CGS21680 (0.1 μM; 94 ± 13%, n = 6) was abolished by the A_{2A} receptor antagonist SCH58261 (30 nM). It is concluded that several purinergic receptors regulate noradrenergic transmission in the rat brain cortex. The $P2Y_1$, $P2Y_6$ and A_1 receptors inhibit EFS-evoked NE release and the $P2Y_1$, $P2Y_{12}$, $P2Y_6$ and A_{2A} receptors increased glial NE uptake.

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P16-16
Interactomic analysis of vdac in rat and bovine mitochondria in neuronal cells

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Two types of binding sites for hexokinase, designated as Type a or Type b sites, have been shown to coexist on brain mitochondria. Hexokinase bound at Type a sites is released by treatment of mitochondria with glucose 6-phosphate (Glc-6-P), while hexokinase bound at Type b sites is refractory to treatment with Glc-6-P. The ratio of type a:type b sites varies with brain mitochondria from different species, this ratio being approximately 90:10, 60:40, 40:60, and 20:80 for mitochondria from rat, rabbit, bovine, and human brain, respectively.

Hk1 attaches by reversibly binding to the OMM protein, porin, also known as the voltage dependent anion channel (vdac). Regarding the nature of brain hexokinase binding sites, in this study, we wished to understand if the differential expression of vdac1 and vdac2 verified between rat and bovine neuronal cells, was linked to distinct vdac interactomes. We approached this question by BN-PAGE of mitochondria treated with dodecyl maltoside enabling the efficient but mild solubilization of mitochondrial membranes. After BN-PAGE, a second-dimensional SDS-PAGE was performed to separate polypeptides as components of vdac complex. These components were identified by mass spectrometry.

Our results are consistent with the possibility that the species-dependent ratio of the Type a: Type b sites is due to differential vdac interactions in bovine and rat neuronal cells.

P16r-17
Modulation of adenosine, dopamine and glutamate receptors in human brain of Alzheimer's disease

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Alzheimer's disease (AD) is the major cause of dementia in the elderly. It is characterized by a progressive memory loss, deterioration in cognitive functions, appearance of senile plaques, neurofrillary tangles and selective neuronal loss in several brain regions. Previous studies have shown modifications of adenosine and glutamate receptors in Frontal Cortex in relation to the progressive stages of AD. The aim of the present work was to study the levels of dopamine D_2, metabotropic glutamate mGlu5, adenosine A_{2A} and A_1 receptors in Parietal and Temporal Cortex from AD cases as compared with non-demented samples used as controls. These receptors were analyzed by radioligand binding assays. An increase in adenosine A_{2A} receptors which correlated well with the progression of disease was observed in both Temporal and Parietal Cortex. In contrast, the adenosine A_1 receptor decreased in AD as compared with controls, without significant correlation to the progression of the disease. On the other hand, the mGluR5 receptor was also decreased in both areas analyzed with lowest levels at highest stages of disease. The expression levels of D_2-like receptors in Parietal and Temporal Cortex in AD subjects shows an increase as compared with non-demented subjects. However, D_2 receptors didn't suffer significant changes. Therefore, the ratio of D_2/D_2-like receptors is decreased in AD patients in both Temporal and Parietal Cortex. Results presented herein show that protein levels of A_1 and A_{2A} adenosine, D_2-like dopamine and metabotropic glutamate mGlu5 receptors are modulated in Parietal and Temporal Cortex of AD patients, being this modulation dependent on the progression of disease and suggest these receptors as promising targets for diagnostic and therapy of Alzheimer's disease.

P16-18
Mutations in presenilin-1 gene linked with Alzheimer's disease cease impairments in calcium channels functions in neurons

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Familial Alzheimer's disease (FAD) mutations in presenilin-1 (PS1) gene have been linked to Ca^{2+} signaling abnormalities. The cleaved PS1 are well known as catalytic component of a gamma-secretase, which cleaves the amyloid precursor protein (APP) and releases the amyloid beta-peptide. In addition uncleaved PS1 function as passive ER Ca^{2+} leak channels controlling steady-state ER Ca^{2+} levels. It was found that many FAD mutations in PS1 gene result in loss of ER Ca^{2+} leak function independently of the gamma-secretase activity. PS1-M146V and PS1-ΔE9 mutants have been shown to have loss and gain of ER Ca^{2+} leak channel function respectively. To determinate the influence of FAD presenilins mutations on SOC channels we performed a series of patch-clamp experiments in whole-cell mode and series of Ca^{2+} imaging experiments with Fura2-AM. The SOC channels activity was decreased in triple transgenic mice hippocampal neurons (3XTg mice; KI-PS1M146V, Thy1-AP-PKM670/671NL, Thy1-tauP301L) comparing to WT mice. The effect of PS1-M146V mutant on SOC channels activity was compared with the effect of PS1-ΔE9 mutant in transfected rat hippocampal neurons and human neuroblastoma SK-N-SH cells. In all checked systems PS1-M146V mutant caused the decrease and PS1-ΔE9 mutant led to the increase of SOC channels activity. The effect wasn't depended on the way of store depletion and was the same for passive store depletion and activation of IP3 pathway. Furthermore the effect wasn't ceased by changes in expression levels of basic determinants of SOC such as STIM1, STIM2, Orai1 and TRPC1. ER Ca^{2+} level was increased in PS1-M146V expressing hippocampal neurons and decreased in PS1-ΔE9. Therefore, SOC channels activity impairments in FAD linked to disrupted Ca2+ leak channel function of PS1.

P16r-19
2,3,7,8-tetrachlorodibenzo-p-dioxin disrupts intracellular calcium homeostasis in human neuronal cell line SHSY5Y

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The persistent xenobiotic agent 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces neurotoxic effects that alters neurodevelopment and behavior both during development and adulthood. There are many ongoing efforts to determine the molecular mechanisms of TCDD-mediated neurotoxicity, the signaling pathways involved and its molecular targets in neurons. In this work, we have used SHSY5Y human neuroblastoma cells to characterize the TCDD-induced toxicity. TCDD produces a loss of viability (EC_{50} = 292 ± 28 nM) linked to an increased caspase-3 activity, PARP-1 fragmentation, DNA laddering, nuclear fragmentation and hypodiploid (apoptotic) DNA content, in a similar way than staurosporine, a prototypical molecule of apoptosis induction. In addition, TCDD produces a decrease of mitochondrial membrane potential and an increase of intracellular calcium concentration. Finally, based on the high lipophilic properties of the dioxin, we test the TCDD effect on the membrane integrity using sarcoplasmic reticulum vesicles as a model. TCDD produces calcium efflux through the membrane and an anisotropy decrease that reflects an increase in membrane fluidity. Altogether these results support the hypothesis that TCDD toxicity in SHSY5Y neuroblastoma cells provokes the disruption of calcium homeostasis, probably affecting membrane structural integrity, leading to an apoptotic process.

P16-20
Astrocytes rescue of tau-related cell death

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Intracellular tau protein aggregates constitute one of the major neuropathological hallmarks of Alzheimer's disease and other tauopathies. The identification of mutations in the Tau gene in familial forms of frontotemporal dementia with parkinsonism linked to chromosome 17 has shown the link between tau and

neurodegeneration and it has made possible to produce lines of genetically modified mice expressing human tau with a pathogenic mutation. We have investigated the characteristics of tau pathology in a transgenic mouse expressing human tau with a P301S mutation (Allen et al., 2002). This transgenic mouse line shows some of the major features of human tauopathies, including progressive accumulation of phosphorylated tau aggregates in brain and spinal cord and cortical neurodegeneration. Recently, we have shown that neuronal precursor cell transplantation is able to rescue the tau-induced neuronal death observed in superficial layers of the cerebral cortex in 5-month old P301S mice (Hampton et al., 2010). Surprisingly, neuronal precursor cells differentiate into astrocytes following transplantation, and exert their effect without migrating from the site of injection, suggesting that such positive effect could be due to released factors rather than to direct cell-cell contact. The effect is reproduced by direct transplantation of stem cells-derived astrocytes confirming that astrocytes are the rescuing agent. The aim of our project is to study how the transplanted astrocytes rescue neuronal death while endogenous astrocytes, although increased in number, are not neuroprotective. In particular we aim to: (i) identify possible factors released by the transplanted astrocytes that could diffuse toward the site of neurodegeneration and promote neuronal survival and (ii) determine whether the origin of the transplanted astrocytes in terms of age and genetic background of the donor animal is relevant. The identification of the neuroprotective factors responsible for the astrocytes-induced neuroprotection will provide important clues for the design of novel therapies for untreatable neurodegenerative diseases such as tauopathies.

P16-21
Superoxide dismutase (SOD) and nuclear factor kB (NFkB) are involved in the molecular mechanisms of homeostatic activity of hemorphins in response to endotoxin-induced stress

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Earlier it has been shown that hemorphins (LVV-hemorhin-7 and hemorphin-7) recovered increased calcineurin activity in response to endotoxin-induced stress. Because SOD and NFkB are partners of calcineurin involved in the molecular mechanisms underlying pathophysiology of different diseases (stress, diabetes, cancer), therefore the goal of present work was to study the influence of mentioned hemorphins on SOD and NFkB activity in endotoxin-induced stress. SOD activity in plasma and brain tissue of rats was measured using SOD Assay Kit-WST (Dojindo Molecular Technologies, Inc., USA). NFkB activity was measured by NF-κB p65 ELISA Kit (Imgenex Corporation, USA). Results obtained demonstrated that hemorphins recovered plasma and brain SOD activity decreased (by 33% in plasma and by 15.2% in the brain) in stress conditions. In the same time it has been found out that hemorphins also act as homeostatic agents in the regulation of cytoplasmic and nuclear NFkB (p65) activity, which was differently expressed in brain tissue cytoplasmic and nuclear fractions in response to endotoxin-induced stress. Earlier it has been shown that hemorphins induce the upregulation of expression level of ferritin heavy chain (Barkhudaryan N. et al., 2008), which is an essential mediator of the antioxidant and protective activity of NF-kB (Bubici C. et al., 2004) and mitochondrial enzyme Mn^{2+}, superoxide dismutase (MnSOD), which is considered as a partner of ferritin. MnSOD in its turn has been identified as a partner of calcineurin (Tokheim A.M, et al., 2006), the main target of hemorphins in the brain and immune system (Barkhudaryan et al., 1992, 2002).

P16-22
Inflammation in Alzheimer's disease and the recover by cannabinoids

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Many studies have shown the relationship between inflammation and Alzheimer's disease (AD). The hallmark in AD is both accumulation of beta-amyloid (Aβ) plates and the presence of TAU protein inside neurons. Furthermore, glial cell activation, occurs after plates appear in brain damaged, producing astrogliosis and microglia activation. Our group has shown inflammation in astrocytes in primary culture comparing Aβ with control cells. Here we determined the action of cannabinoids in Aβ inflammation in astrocytes in culture. Protein expression levels were detected by western-blot and ELISA techniques in astrocytes in primary culture treated with Aβ and/or cannabinoids. Using Aβ (10 μM) during 24 hour, an increase of pro-inflammatory mediators (NFκB, TNF-α and IL-1β), compared with control astrocytes was detected. Treatment with Win 55, 212-2 (10 μM) produced increase of anti-inflammatory mediators (PPAR-γ) and decrease of pro-inflammatory mediators, such as NFκB, TNF-α and IL-1β, protecting cells to the toxic action of Aβ. In transgenic mice (APP/Preseniline I), using microarray to principal inflammatory proteins, we detect induction of pro-inflammatory mediators and reduction of anti-inflammatory mediators compared with wild type mice. With those results we demonstrate an unbalance between inflammatory and anti-inflammatory mediators in Alzheimer's disease and a possible positive effect of cannabinoids in AD.

P16r-23
Alterations in GRK, cholesterol and desmosterol levels in rats with chronic-relapsing experimental allergic encephalomyelitis and effects of an ethanolamine phosphate salt

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Multiple sclerosis (MS) is an autoimmune-mediated neurodegenerative disease with characteristic foci of inflammatory demyelination and oxidative stress. Chronic-relapsing experimental allergic encephalomyelitis (CR-EAE) is characterized by neuropathological and immunological dysfunctions which closely resemble those found in MS and is used as an animal model of MS. Ethanolamine plasmalogens are known to be powerful antioxidants that lower membrane oxidizability. Previous studies from our group demonstrated a decrease in somatostatin binding to its receptors in hippocampal membranes from rats with CR-EAE. To better comprehend the underlying mechanism of this decrease, in the present study we measured the levels of GRK 2, 5 and 6, cholesterol, desmosterol and β-arrestins in hippocampal membranes from CR-EAE rats. In addition, we analyzed the effects of a calcium, magnesium and phosphate ethanolamine salt (EAP) on these parameters. CR-EAE induced in Lewis rats decreased GRK2, GRK6 and desmosterol levels and increased

cholesterol and β-arrestin levels in hippocampal membranes. EAP administration (600 mg/kg) 2 days prior to CR-EAE induction prevented the changes found in the above mentioned parameters. The present results suggest that the decrease in the hippocampal somatostatin binding parameters is partly due to the decrease in membrane GRK levels and the increase in β-arrestins, favoured by the increased membrane cholesterol content. The effects of EAP on these parameters is possibly due to its antioxidant role at the membrane level.

P16-24
Dopamine D2 and 5-hydroxytryptamine 5-HT(2A) receptors assemble into functionally interacting heteromers and their relevance for treatment of schizophrenia

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In view of the co-distribution of dopamine D(2L)R and 5-hydroxytryptamine 5-HT(2A) receptors (D(2L)R and 5-HT(2A)R, respectively) within inter alia regions of the dorsal and ventral striatum and their role as a target of antipsychotic drugs; in this study we assessed the potential existence of D(2L)R-5-HT(2A)R heteromers in living cells and the functional consequences of this interaction. Thus, by means of a proximity-based bioluminescence resonance energy transfer (BRET) approach we demonstrated that the D(2L)R and the 5-HT(2A)R form stable and specific heteromers when expressed in HEK293T mammalian cells. Furthermore, when the D(2L)R-5-HT(2A)R heteromeric signaling was analyzed we found that the 5-HT(2A)R-mediated phospholipase C (PLC) activation was synergistically enhanced by the concomitant activation of the D(2L)R as shown in a NFAT-luciferase reporter gene assay and a specific and significant rise of the intracellular calcium levels were observed when both receptors were simultaneously activated. Conversely, when the D(2L)R-mediated adenylyl cyclase (AC) inhibition was assayed we showed that costimulation of D(2L)R and 5-HT(2A)R within the heteromer led to inhibition of the D(2L)R functioning, thus suggesting the existence of a 5-HT(2A)R-mediated D(2L)R trans-inhibition phenomenon. Finally, a bioinformatics study reveals that the triplet amino acid homologies LLT (Leu-Leu-Thr) and AIS (Ala-Ile-Ser) in TM1 and TM3, respectively of the D2R-5-HT(2A)R may be involved in the receptor interface. Overall, the presence of the D(2L)R-5-HT(2A)R heteromer in discrete brain regions is postulated based on the existence of $D_{(2}L)R$-5-$HT_{(2}A)$ receptor-receptor interactions in living cells and their codistribution inter alia in striatal regions. Possible novel therapeutic strategies for treatment of schizophrenia should be explored by targeting this heteromer.

P16-25
Metabolism of [1,6-^{13}C] glucose in glutamatergic and GABAergic compartments *in vivo* in the rat brain

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Energy metabolism supports both inhibitory and excitatory neurotransmission processes. Using dynamic high resolution ^{13}C magnetic resonance spectroscopy (MRS) *in vivo*, we now investigated intermediary metabolic pathways contributing to γ-aminobutyric acid (GABA) synthesis and GABAergic neurotransmission in the rat brain. Male Sprague-Dawley rats (267 ± 9 g, n = 8) under α-chloralose anesthesia were infused with [1,6-^{13}C]glucose, and ^{13}C MRS was performed at 14.1 T as previously described [Duarte et al. (2011) Front Neuroenergetics 3, 3]. Enhanced sensitivity and spectral resolution achieved at high magnetic field, allowed quantifying, for the first time, incorporation of labeling into the three aliphatic resonances of GABA *in vivo*. Metabolic fluxes were determined with a newly designed mathematical model of brain metabolism that included glial, glutamatergic and GABAergic compartments. GABA synthesis rate was 0.10 ± 0.01 μmol/g/min. Apparent GABAergic neurotransmission, i.e. GABA-glutamine cycle, accounted for 20% (0.043 ± 0.002 μmol/g/min) of the total neurotransmitter cycling between neurons and glia. Total brain glucose oxidation was 0.45 ± 0.02 μmol/g/min, from which 40% and 8% occured in the tricarboxylic acid (TCA) cycles supporting glutamate-glutamine and GABA-glutamine cycles, respectively. The remainder fraction of glucose oxidation was found to take place in glia, including a contribution of 19% from pyruvate carboxylation (0.072 ± 0.004 μmol/g/min). Like in previous studies, exchange fluxes from 2-oxoglutarate to glutamate were in the same order of the respective TCA cycles in the three compartments. In conclusion, the present results show a substantial metabolic activity in GABAergic neurons and, furthermore, emphasize the active role of glial oxidative metabolism in supporting neurotransmission in both glutamatergic and GABAergic neurons.

P16r-26
Study of the effect of long-term caffeine treatment in p15 rat brain

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Caffeine is the most widely consumed psychoactive substance in the world. Nearly 70% of women take products containing caffeine (coffee, tea, soft drinks, chocolate, and some medications) during pregnancy. Although several clinical investigations have shown that high caffeine intake during pregnancy represent a danger in offspring, its effect on the adenosinergic system in different brain regions has been less studied. In these experiments, we analyze adenosine receptors (A_1 and A_{2A} subtypes) and GFAP-ir on different brain regions including Cingulate cortex (Cg2), Dentate Gyrus (GD), medial Preoptic area (mPOA), Secondary Somatosensory cortex (S2) and Striatum (Str). For this purpose, we used postnatal day 15 animals whose mothers consumed 1 g/l caffeine (C) or tap water (W) during gestation and lactation, and we established four experimental groups: W-W, W-C, C-W and C-C. In order to analyze the motor, cognitive function and working memory, a homing test, examining offspring's nest-seeking behavior, was performed on postnatal day 13. Immunohistochemical and Western blotting analysis of samples were performed at postnatal day 15. Our results in behavioral test showed that latency to reach the home bedding area was significantly higher in those p13 animals from mothers treated with caffeine during gestation and lactation. Also, we have demonstrated that caffeine consumption in these periods induces significant changes in immunoreactivity depending on the analyzed region. For example, in Cg2 a significant increase of A_1 and GFAP-ir was observed, while no changes were found in Str. Our results suggest that chronic caffeine exposure during

gestation and lactation possibly implies a reorganization of neural circuits and a modulation of adenosine system.

P16-27
The neurotrophin-binding domain of the human neurotrophin receptor as a specific cell surface target for tetanus toxin

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Tetanus toxin (TeTx) is a potent neurotoxin, lethal in a subnanomolar range, which acts on peripheral and central nervous systems causing spastic paralysis. TeTx is internalized at presynaptic membrane of motorneurons. Then, it is retroaxonally transported to the spinal cord where blockage of neurotransmitter release occurs, resulting in characteristic motor spasms. The C-terminal domain of the heavy-chain of tetanus toxin (Hc-TeTx) is responsible for nerve cell recognition, binding and internalization. The specific mechanism by which the toxin binds and enters the nervous cell has not been elucidated yet, although the role of gangliosides as high-affinity receptors for TeTx has been described. However, current research results support a dual-receptor mechanism where proteins of the cell surface may be involved in toxin binding, thus explaining its extremely high affinity and specificity. Based on previous studies, we propose Trk receptors as possible targets for TeTx cell-surface binding. Molecular modeling analysis allows us to study how Hc-TeTx mimics the binding of neurotrophin-4/5 to the d5 ectodomain of neurotrophin receptor TrkB. We predict certain sequences in Hc-TeTx responsible for its interaction with TrkB and directed mutagenesis in three amino-acidic positions was performed. The mutant protein tested in the cerebellar granule neurons model shows a decrease in the TrkB activation, when compared with that caused by Hc-TeTx wild-type, thus indicating the probable implication of this position in the interaction, although it seems that there would be more residues involved in this interaction. The determination of the TeTx receptor will be a major breakthrough that will have important implications in designing new drugs capable of crossing the blood–brain barrier.

P16r-28
Low doses of dimethoate alters cardiolipin metabolism and induced apoptosis in rat brain

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The widely used insecticide, dimethoate (D), was administered for 5 weeks (subchronic) at low dose (15 mg/kg) to male Wistar rats with the aim to simulate the exposure to pesticide residues in food. In brain, D administration provoked oxidative stress, increased the expression of caspase-3 in substantia nigra (SN) and the expression and activity of mili- and micro- calpains in cerebral cortex (CC) and SN, we also found a higher ratio Bax/Bcl-2 and a significant release of cytochrome C (CytC) from mitochondria (M) in both brain regions compared to controls. D also affect the inner mitochondrial membrane integrity reducing the electrochemical potential between 30% and 60% compared to controls; this effect correlates with a decrease of mitochondial cardiolipin (CL) from 30% to 50% and with an increase of mono-lyso CL (MLCL) between 25% and 40%. CL is rich in polyunsaturated fatty acids (PUFAs), the composition varies greatly within brain regions. The decrease could be associated to the oxidative damage of their fatty acyl chains, D-treatment caused an increase of 18:0 and a decrease of PUFAs (18:2, 20:4, 22:5, 22:6) in CL and MLCL (except the ratio 18:0/18:1 which is higher in MLCL). These changes could be due to the remodeling action of PLA2/AcilCoA-Acetilase on the peroxidated CL pool and could explain the D-dependent decrease in CL content and also the release of CytC from M. Both effects were observed mainly in SN. Since the close relationship between, the decrease of CL and the induction of apoptosis, we proposed that D-induced oxidation of CL fatty acids could be at least one of the toxic mechanisms of D as an environmental pollutant. The results could contribute to understand the etiological role that pesticide residues in food could play in neurodegenerative diseases.

P16-29
Action of β-N-methylamino-L-alanine on both GSK3 and TDP-43 in human neuroblastoma

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β-*N*-methylamino-L-alanine (L-BMAA) is a neurotoxic amino acid that has been related to various neurodegenerative diseases. Taking into account that both glycogen synthase kinase-3 (GSK3) and (TAR)-DNA-binding protein 43 (TDP-43) have been described as possible neurodegenerative disease hallmarks, the main goal of this study was analyze whether the treatment with L-BMAA produces alterations in both biomarker proteins.

The experiments were performed in human neuroblastoma cells SH-SY5Y. In all experiments, cells were incubated for 24 hour with 10 mM L-BMAA. Western blots showed a discrete increase in GSK3 α/β in cellular total extracts. When the nuclear fraction was analyzed, a significant increase in GSK3β was observed, without appreciates any decline in the levels of these proteins in the cytosol. GSK3β has been established as an antiapoptotic regulator of death receptor-induced extrinsic apoptosis, but this protective action occurs when GSK3β is located in the cytosol. Under basal conditions, GSK3β is mostly cytosolic and associated with multiprotein complexes, which are their substrates. After stimulation, GSK3β is released from protein anchors and it is translocated to nucleus, where it is highly active and has been involved in important cellular functions achieved by GSK3 phosphorylation of numerous transcription factors and produces cellular death. Therefore, our results indicate that the toxicity of L-BMAA implies both an increase of GSK3β synthesis and a GSK3β translocation to the nucleus, as part of its action producing cell death. Our results also indicated that L-BMAA cause an increase in TDP-43 and alterations in its structure, giving rise to the formation of aggregates of TDP-43 without nuclear TDP-43 depletion. This study demonstrated that L-BMAA leads the increase of GSK3 and TDP-43, two possible biomarkers for neurodegenerative diseases.

P16r-30
Trafficking and function of juvenile NMDA receptors

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NR3A and NR3B are non-conventional members of the NMDAR subunit family that modulate NMDAR-dependent synaptic plasticity by altering the calcium permeability and magnesium sensitivity of the receptor complexes. NR3A subunits are highly expressed in the brain during critical periods of postnatal development, but down-regulate sharply afterwards and expression remains attenuated into adulthood. Intriguingly, a number of activity-dependent pathways converge in modulating NR3A expression and trafficking. Because of their remarkable subunit specificity, they promote the replacement of non-conventional NMDARs (NR1/NR2/NR3A heteromers) with mature subtypes (NR1/NR2 heteromers) that flux calcium in a coincident manner. Removal and replacement of juvenile NR3A-containing NMDARs is essential for the development of strong NMDAR currents, full expression of synaptic plasticity, a mature synaptic organization characterized by more synapses and larger postsynaptic densities, and the ability to consolidate memories.

These results strongly suggest that NR3A acts as a molecular brake to prevent the premature strengthening and stabilization of excitatory synapses. Importantly, reactivation of non-conventional NR3A expression occurs in brain pathologies characterized by spine dysfunction and loss. Specifically, I will present recent findings indicating that aberrant NR3A expression underlies synapse loss and motor and cognitive decline in Huntington's disease, and discuss the underlying mechanisms in the context of developing targeted therapies.

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P16-31
Knocking down of substance-P and α-CGRP modulates inflammatory sensitization of TRPV1

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Transient receptor potential vanilloid 1 (TRPV1), a polymodal ion channel predominantly expressed in dorsal root ganglion neurons (DRGs), senses distinct nociceptive stimuli acting as a major integrator of inflammatory pain. Under inflammation, TRPV1 activity is greatly potentiated leading to thermal hyperalgesia. TRPV1 inflammatory sensitization involves both the modification of channel gating by covalent modification and the recruitment of channels to the neuronal surface. Mobilization of new channels to the membrane of a subpopulation of DRG neurons occurs through SNARE-dependent exocytosis. A question that emerges is which is the mechanism involved in TRPV1 mobilization in nociceptors? A plausible hypothesis is that the recruitment of channels occurs in the so-called peptidergic nociceptors that contain the pro-inflammatory peptides substance P (SP) and α-CGRP in large dense core vesicles (LDCVs). We have investigated the contribution of SP and α-CGRP on modulating TRPV1 sensitization in rat and mice DRG neurons in culture. The involvement of SP and α-CGRP was assessed by inhibiting their expression using siRNA in rat DRG neurons and in a double SP and α-CGRP knock-out mouse. Using confocal and electron microscopy, we found that TRPV1 co-localizes with α-CGRP in LDCVs. Knocking down the expression of SP and α-CGRP resulted in reduced surface expression of TRPV1 upon exposure to pro-inflammatory mediators. Functional analysis using calcium fluxes and patch clamp electrophysiology, was consistent with a decreased inflammatory sensitization of TRPV1. These data support the tenet that SP and α-CGRP are crucial in modulating TRPV1 sensitization and could be a potential target in treating inflammatory pain.

P16-32
Gene expression profile of the insulin signaling pathway in APP/PS1 transgenic mice as a model for alzheimer disease

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Introduction: Obesity, metabolic syndrome and insulin-resistance in type 2 diabetes mellitus (T2DM) are risk factors for Alzheimer's disease (AD). Indeed, it is well known, the association between obesity and altered signalling mechanisms dependent of insulin, and that implies a greater susceptibility to neurodegenerative processes. Besides, *in vivo* studies with obese mice show the appearance of alterations characteristic of neurodegeneration observed in AD. In these cases, it appears that peripheral hyperinsulinemia and insulin resistance are the determining factors in the increased risk of developing AD. In fact, AD is considered by some authors as a form of diabetes 3. Thus, the observed insulin signaling impairment in the cortex and hippocampus of AD models suggest the interest to study in deep this pathway to a better understanding of the disease. The main purpose of the present research was to analyze changes in the transcriptional activity of genes related with the insulin signaling pathway in the double transgenic APP/PS1 mice, a well-known model of AD.
Material and methods: To analyze gene expression we used a PCR array system (Insulin Signaling Pathway PCR Array Gene Expression, PAMM-030Z Quiagen). Samples from cortex and hippocampus from both, wild type C57BCL/6J and APP/PS1 (APPswe, PSEN1dE9) mice, were mechanically homogenized using a potter-elvehjem homogenizer. Total RNA was extracted and purified using Trizol (Quiagen). The RNA concentration of samples was quantified using the Nanodrop and RNA integrity was determined using Bioanalyzer. PCR arrays were performed on samples from 3, 6, 9 and 12 months-old mice. The up- and down-regulated genes were further validated by single Real-time PCR analysis and the protein content was determined by Western blot using standard procedures in a Bio-Rad Mini-Protean system.
Results and conclusion: A total of 20 genes show significative changes in transcriptional activity. Among them, leptin and prolactin resulted down-regulated in aged mice. The preliminary results show the involvement of AMPK pathway, which is related to sterol regulatory element binding protein (SRBP's), energy balance and memory processes in the APP/PS1 mice model of AD.

P16-33
The effect of tandospirone on expression of NMDA receptor subunits and cognitive function

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The glutamatergic neurotransmitter system has been sugested as playing an important role in the aetiopathogenesis of many neuropsychiatric deseases, specially in schizophrenia, based on

findings on various aspects of neural substrates ranging from molecular interactions to the neuronal network in the human brain (Goff and Coyle 2001, Kristiansen et al., 2007). Tandospirone, an azapirone, is a selective 5-HT1A receptor partial agonist and displays approximately 60% of the effect of the full agonist 8-OH-DPAT. We have studied the effect of repeated administration of tandospirone (0.05 and 5 mg/kg) on expression of *N*-methyl-D-Aspartate (NMDA) receptor subunit by imunoblotting. We found that tandospirone increased the expression of GluN2B subunit and decreased the expression of GluN1 subunit in frontal cortex. In hippocampus tandospirone (only the dose 5 mg/kg) decreased the expression GluN2B subunit. NMDA receptors are necessary for a formation of memory and information processing and therefore we evaluated the effect of tandospirone in several cognitive tasks and prepulse inhibition of acoustic startle response in rats. Anxiolytic properties of tandospirone, as marketed in Japan, have been demonstrated in human and animal. We found that tandospirone impaired cognitive function in our selected tasks except Novel object recognition test and improved anxiolytic behavior (shock-induced ultrasonic vocalization). We have suggestive that changes in NMDA subunits expression after administration of tandospirone are responsible for observed cognitive impairment in rats and that the utilization of tandospirone in schizophrenic patients is controversial.

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P16-34
Amylin increases hippocampal GSK3β phosphorylation (Ser 9) by leptin

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Introduction: Leptin (Lep), an adipose-derived hormone, exerts very important functions in the body mainly on energy storage and availability to the brain. Although the major interest in Lep are based mainly in its role in the regulation of energy balance since resistance to leptin effects is the primary risk factor for obesity, more recent investigations also show their effects on brain cognition and neuroprotection. Furthermore, It appears that peripheral hyperinsulinemia and insulin resistance are determining factors in the increased risk of developing neurodegenerative diseases like Alzheimer. Leptin activates multiple signaling pathways in neurons, including PI3K/AKT pathway which indicates a degree of cross-talk between cell survival and neuroprotection. We investigated the potential neuroprotective effects of leptin synergy with two molecules that migth contribute to their neuroprotective effects as amylin (Amy) and metformin (Met).

Material and methods: Leptin, amylin and metformin, alone or in combination were administered to adult C57BL/6J mice. One group received 1 mg/kg Lep and 50 μg/kg Amy intraperitoneally, followed by 300 mg/kg oral Met administration. Another group recived the same doses but one hour later 24.5 mg/kg of kainic acid (KA) intraperitoneally to cause damage in the hippocampus (CA1). Mice were sacrificed 24 hours after KA injection, and hipocampi were studied by western-blot and histological analysis.

Results and conclusion: The combination of leptin and amylin causes an increase in the phosphorylated, inactive, form of GSK3-β (Ser 9), and a concomitant decrease in the phosphorylated forms of downtream targets like Cyclin D and cdk4. We also studied the effects of combinated treatments with Lep and Amy in the expression of the serine/threonine protein kinase AKT and β-catenine. As long as GSK3-β is directly related to Tau hyperphosphorylation in Alzheimer's disease, and in other tauthopathies, these results show the neuroprotective effects of Lep and Amy in relation with AD.

P16-35
Patient-derived olfactory mucosa stem cells as a model for Friedreich's ataxia

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Friedreich's ataxia (FA) is the most common hereditary ataxia in the Caucasian population. FA is caused by autosomal recessive mutations that produce a deficiency in frataxin, a protein which is thought to play an important role in the regulation of mitochondrial function. FA is characterized by a severe neurological deterioration resulting from neurodegeneration which predominantly affects the dorsal root ganglia, spinal cord, brainstem and cerebellum.

In order to obtain a cell model for FA, we have isolated stem cell lines from biopsies of the olfactory mucosa of FA patients and healthy donors. These olfactory mucosa stem cells (OMSCs) are neural crest-derived mesenchymal stem cells with the ability to differentiate into neuron and glial-like cells, among other cell types.

OMSCs from FA patients exhibit typical features associated with frataxin deficiency including increased oxidative stress and reduced aconitase activity. Furthermore, OMSCs from FA patients have increased p53 levels. Our results are consistent with the view that OMSCs provide a good cell model system to study the molecular consequences of frataxin deficiency in patient-derived neural tissue cells.

P16-36
Autophagy disregulation in the retina: neurodegeneration and aging

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Accumulation of undegraded proteinaceus material represents a hallmark in many retinopathies and in aged-related macular degeneration, major cause of blindness in the elderly population. Autophagy contributes to maintain cellular homeostasis through the removal of damaged cellular components in lysosomes. Although alterations in autophagic function underlie the basis for a growing number of human diseases, very little is known about the contribution of this quality control mechanism to the pathophysiology of the retina. We found that autophagic flux is less efficient in the aged retinas in basal and under starvation

conditions associated to increases of lipofuscin, ubiquitinated proteins and p62 levels in all retinal layers from old C57B6/J mice. Decreased expression of the the mRNA expression of the autophagy regulator *Beclin1*, main component of the initiation complex, and of *Atg7,* the limiting enzyme for the elongation of the autophagosome limiting membrane was observed in the aged mice. We then analyzed the consequences of macroautophagic blockage in retinal quality control by crossing the conditional Atg5 knockout animals ($Atg5^{flox/flox}$) with the *nestin-Cre* mice. We observed apoptotic nuclei stained with TUNEL in the retina from $Atg5^{flox/flox}$*; nestin-Cre* mice. Moreover, we found that at 7 weeks of age $Atg5^{flox/flox}$*; nestin-Cre* mice manifested a clear reduction in the visual function as determined by electroretinogram (ERG). Interestingly, and as it has been previously described in aged mice, they displayed statistically significant reduction in the scotopic tests, which are associated to the rod function, but not to the cone-associated parameters. In conclusion, we have identified a primary malfunction of macroautophagy in the aging retina that contributes to the age-associated reduction of visual function.

P16-37
Biochemical characterization of α-synuclein as a stimulator for the GSK-3β-mediated phosphorylation of Tau protein

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Purpose: Tau protein (tau) is the microtubule-associated proteins, which are exclusively expressed in the central nervous system. Tau may play an important role in the neurite outgrowth and axonal transport. In several neurodegenerative diseases, the highly phosphorylated tau forms a paired helical filament (PHF) and the highly phosphorylated tau disrupts neuronal function. The phosphorylated tau is also detected in the brain tissue of α-synucleinopathy caused by abnormal accumulation of α-synuclein (α-SN), which is a causative molecule for familial Perkinson's disease (PD). Therefore, the present study had been done to determinethat (i) the stimulatory effect of α-SN on the GSK-3β-mediated phosphorylation of tau; (ii) the physiological interaction between alpha-SN and tau or GSK-3β; and (iii) the inhibitory effect of Hsp70 on the α-SN-induced phosphorylation of tau by GSK-3β.
Results and Discussion: α-SN stimulated the phosphorylation of tau by GSK-3β and autophosphorylation of the kinase *in vitro*. GST-pull-down assay confirmed that (i) α-SN directly bound to tau as well as GSK-3β; (ii) tau was binding to the C-terminal acidic region; (iii) GSK-3β was binding to the regions containing KTKEGV repeat and NAC domain of α-SN; and (iv) the NAC domain and an acidic region of α-SN are responsible for the stimulation of GSK-3β-mediated phosphorylation of tau. As expected, Hsp70 suppressed the α-SN-induced phosphorylation of tau by GSK-3β through its direct binding to α-SN. Since the expression of α-SN is promoted by oxidative stress, the oxidative stress-induced accumulation of α-SN may induce the high phosphorylation of tau by GSK-3β involved in the neuronal cell dysfunction. In addition, the finding that Hsp70 acts as a suppressor of α-SN, suggests that the cellular level of Hsp70 may be a novel therapeutic target for the α-SN-mediated tau phosphorylation in an initial stage of neurodegenerative diseases.

P16-38
Downregulated expression of genes involved in myelination in knock-out mice lacking Cysteine String Protein-alpha (CSP-alpha)

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Synaptic transmission continually occurring at nerve terminals likely induces use-dependent waning of the molecular machinery involved in synaptic vesicle cycling. Interestingly, knock-out (KO) mice lacking the synaptic vesicle protein Cysteine String Protein-alpha (CSP-alpha) suffer from a presynaptic degeneration that is activity-dependent. For example, at the hippocampus of CSP-alpha KO mice, the terminals from high firing rate GABAergic basket cells preferentially degenerate in contrast to glutamatergic terminals with a lower action potential firing rate (García-Junco-Clemente et. al., JNeurosc. 30:7377-7391, (2010)). However, the molecular mechanisms responsible for presynaptic degeneration are not well known. Interestingly, mutations in DNAJC5, encoding CSP-alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis in humans (Nosková et al., Am J Hum Genet. 89:241-52 (2011)). Here, we have investigated gene expression changes secondary to CSP-alpha genetic deletion at hippocampi isolated from CSP-alpha and control littermates at 20–24 days postnatal age. We have used two gene expression analysis methods to study transcriptional changes at the hippocampus of CSP-alpha KO mice: (i) Affymetrix Mouse Genome 430 2.0 Arrays for genome-wide expression analysis and (ii) competitive PCR combined with MALDI-TOF mass spectroscopy analysis of PCR products (Sequenom). Both methods indicate an unexpected downregulated expression of several genes related to myelination in the hippocampus of CSP-alpha KO mice. Consistently, biochemical and electron microscopy analysis detect signs of reduced myelination in CSP-alpha KO mice. Future experiments will have to elucidate the unexpected relationship between the genetic removal of CSP-alpha, the dysregulation of myelination and the synaptic degeneration in CSP-alpha KO mice.

P16-39
Src kinase plays a key role in neuroinflammatory processes controlling microglia activation

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Neuroinflammatory processes are usually associated with exacerbated microglia activity. Src is a tyrosine kinase member of the Src family kinase (SFK) that is highly expressed in the CNS. The main goal of this work was to investigate the role of Src on inflammatory processes mediated by microglia. In this regard, we used cultured microglia, lentiviral-mediated gene knockdown, and hypoxia and retinal ischemia-reperfusion (I-R) models.

We found that processes usually associated with increased inflammation, i.e. hypoxia and I-R, strongly enhanced Src activa-

tion in microglia. Furthermore, knocking down the expression of RhoA by a shRNA-based approach led to robust microglia activation and increase in pSrc levels. Such activation was ROCK2-dependent as only ROCK2 shRNA-based knockdown (and not ROCK1) decreased the expression of CSK (the endogenous repressor of Src). Src activity appears also to be central to the expression of pro-inflammatory mediators iNOS and TNF-α, as SKI-1, a selective inhibitor of Src, blocks LPS-induced upregulation of these markers. When RhoA or ROCK-2 was knocked down in N9 microglial cells, NO and TNF-α release was constitutively increased compared to controls. Accordingly, knocking down Src or overexpressing a Src kinase dead mutant (Src K295R) construct in cells in which RhoA and ROCK-2 expression was knocked down, suppressed the increase in NO production and TNF-α release. Overall, we describe a novel regulatory pathway regulating Src-mediated microglia activation. In this pathway RhoA/ROCK-2/CSK endogenously repress Src, to maintain microglia at a resting state. Our results might be important in the context of neurodegenerative processes where overactive microglia directly impacts on neuronal viability.

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P16m-40
LRRK2 directly phosphorylates Akt1 as a possible physiological substrate: impairment of the kinase activity by Parkinson's disease-associated mutations

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Leucine-rich repeat kinase 2 (LRRK2) is the causal molecule for autosomal-dominant familial Parkinson's disease, although its true function, including its physiological substrates, remains unknown. Here, using *in vitro* kinase assay with recombinant proteins, we demonstrated for the first time that LRRK2 directly phosphorylates Akt1, a central molecule involved in signal transduction for cell survival and prevention of apoptosis. Ser473, one of two amino acids essential for Akt1 activation, was the target site for LRRK2. A knockdown experiment using intact cells also demonstrated LRRK2-mediated phosphorylation of Akt1 (Ser473), suggesting that Akt1 is a convincing candidate for the physiological substrate of LRRK2. The disease-associated mutations, R1441C, G2019S, and I2020T, exhibited reduced interaction with, and phosphorylation of, Akt1, suggesting one possible mechanism for the neurodegeneration caused by LRRK2 mutations.

P16-41
Sphingosine-1-phosphate evokes excitation of sensory neurons and nociception in mice and humans

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Sphingosine-1-phosphate (S1P) is an essential bioactive lipid mediator in inflammation. There is evidence that S1P increases the excitability of nociceptors, however, its role in spontaneous inflammatory pain is still unknown. Here, we investigated the effect of S1P on the axon-reflex in human skin, spontaneous pain-like behaviour in mice *in vivo* after its local injectionand the excitatory effect on nociceptive neurons*in vitro*. We examined the axon-reflex in human skin after intracutaneous S1P or vehicle injection by taking repeated laser-Doppler images. S1P induced a dose-dependent flare reaction showing the most pronounced effect at 0.5 and 1 mM. In addition, we performed behavioural studies after S1P injection into the hind paw of wt, $S1P_1^{fl/fl}$ and SNS-$S1P_1^{-/-}$ mice which selectively lack the $S1P_1$ receptor subtype in Nav1.8 expressing nociceptive neurons. S1P evoked flicking and licking behaviour in all three mouse strains. In current clamp experiments, S1P induced depolarization and AP firing in cultured neurons from wt mice. In microfluorimetric Ca^{2+} measurements S1P evoked transient rises in $[Ca^{2+}]_i$ in wt DRG neurons which was not detectable in Ca^{2+} free conditions but currents were still present in cation free solution. Furthermore, the S1P-induced inward current was almost fully abolished by the non-specific Cl^- channel inhibitor niflumic acid suggesting activation of an excitatory chloride current by S1P. Since the S1P-induced inward current was fully prevented in the presence of the G-protein inhibitor, GDP-β-S, suggesting a G-protein dependent mechanism, we performed *in situ* hybridization assays and detected $S1P_3$ receptor mRNA in practically all neurons in the mouse dorsal root ganglia. In line with this finding, S1P-induced depolarization in single neurones and spontaneous pain behaviour *in vivo* was significantly reduced in $S1P_3^{-/-}$ mice. Our data support the idea that S1P produces spontaneous pain-like behaviour via G-protein dependent activation of an excitatory inward current, which seems to involve the activation of $S1P_3$ receptors as well as the G-protein dependent activation of Cl^- channels in sensory neurons.

P16-42
Sex differences in striatal dopamine D1 receptors in adult rats mediated by a chronic postnatal application of methamphetamine

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Methamphetamine (MA) is a powerful stimulant with a potency to cause psychic addiction and with neurotoxic properties. Repeat MA use is an alarming problem both in the Czech Republic and worldwide. Men and women differ markedly with regard to their use of, and responses to MA. Except for *N*-methyl-D-aspartate receptor antagonists, the amphetamines are

the only class of addictive drugs known to be associated with striatal dopamine depletion.

The purpose of this study was to examine the effect of chronic prenatal exposure of MA (5 mg/kg during gestation) or the same volume of saline (S) in combination with chronic administration of MA (1 mg/kg) or S in adulthood on Wistar rats. The effects of MA on striatal dopaminergic system (D1 and D2 receptors estimated by means of [^{3}H]-SCH23390 and [^{3}H]-spiperone specific binding) were evaluated. We compared totally four groups: (i) prenatal and postnatal saline application (S/S); (ii) prenatal saline and postnatal MA (S/MA); (iii) prenatal MA and postnatal saline (MA/S); (iv) prenatal and postnatal MA (MA/MA). We results obtained by means of [^{3}H]-SCH23390 are as follows: (i) we have found the decrease in the specific binding to 72% in female group (S/MA) but the increase to 120% in male group (S/MA) when compared to corresponding controls, (ii) Scatchard analysis revealed that the changes observed in females are associated with a drop in the number of binding sites, on the contrary, those in males with the higher affinity of isotope to its binding site. In conclusion, we have found that the chronic postnatal application of MA evoked the significant sex-dependent changes in D1 receptors in the striatum. Our results are in accordance with the study reporting alterations in dopaminergic neurotransmitter system in human nucleus accumbens after a chronic treatment of amphetamine. Our experiments also suggest that the prenatal exposure of MA probably does not markedly influence striatal D1 receptors. Supported by GACR project (P303/10/0580).

P16-43
Early age-mediated transcriptomal changes in mouse substantia nigra

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Substantia nigra pars compacta (SNpc) is highly sensitive to normal aging and is selectively degenerated in Parkinson's disease (PD). Until now, molecular mechanisms behind SNpc aging have not been fully investigated using high throughput techniques. Here, we show early signs of aging in SNpc, which are much more obvious than in ventral tegmental area (VTA), a region adjacent to SNpc but less affected in PD. Aging-associated early changes in transcriptome were investigated comparing late middle-aged (18 months old) to young (2 months old) mice in both SNpc and VTA. A meta-analysis of published microarray studies resulted in the generation of a common 'transcriptional signature' of the aged (24 months old) mouse brain. SNpc of late-middle aged mice shared characteristics with the transcriptional signature, suggesting an accelerated aging in SNpc. Age-dependent changes in gene expression specific to SNpc were also observed, which were related to neurological functions and inflammation. As a proof of principle, the role of two age-regulated genes in the normal function of SNpc cells are being studied in detail. This study will aid to understand the mechanism of SNpc aging and its potential contribution to age-related disorders like PD.

P16r-44
Reduced connexin43 expression correlates with c-Src activation, proliferation and glucose uptake in reactive astrocytes after an excitotoxic insult

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In diverse brain pathologies, astrocytes become reactive and undergo profound phenotypic changes. Connexin43, the main gap junction channel-forming protein in astrocytes, is one of the proteins modified in reactive astrocytes. Down-regulation of Cx43 in cultured astrocytes activates c-Src, promotes proliferation and increases the rate of glucose uptake, however, so far there have been no studies examining whether this cascade of events is taking place in reactive astrocytes. In this work, we analyzed this pathway after a cortical lesion induced by a kainic acid injection. As previously described, astrocytes reacted to the lesion with an increase in GFAP and a decrease in Cx43 expression. Some of these reactive astrocytes proliferated, as estimated by BrdU incorporation and cyclins D1 and D3 up-regulation. In addition, the glucose transporter GLUT-3 and the enzyme responsible for glucose phosphorylation, type II hexokinase, were induced in reactive astrocytes, suggesting an increased glucose uptake. Previous *in vitro* studies reported that c-Src is the link between Cx43 and glucose uptake and proliferation in astrocytes. Here we found that c-Src activity was up-regulated in the lesioned area suggesting that c-Src mediates the effect of Cx43 on glucose uptake and proliferation in reactive astrocytes after an excitotoxic insult. Interestingly, in this study we identify c-Src, GLUT-3 and Hx-2 in the signalling mechanisms involved in the reaction of astroglia to injury. Altogether these data contribute to identify new therapeutical targets to enhance astrocyte neuroprotective activities.

P16-45
Influence of cytosolic prion protein on function of microtubule-associated proteins

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According to our previous studies, prion protein (PrP) may bind to tubulin leading to its oligomerization. This interaction influences microtubule formation and derange tubulin function. Neurotoxicity of cytosolic form of PrP, reported in prion diseases, may be directly related to PrP-tubulin binding.

Division spindles are dynamic structures in which precise cooperation of microtubule-associated proteins (MAPs) and microtubules plays a key role. In this report we have shown that cells treated with membrane-penetrating PrP peptide (PrP residues 1-30) generate aberrant mitotic spindles. Improper morphology of division spindles may result both from altered microtubule dynamics and impaired function of molecular motors. To shed light on the mechanism of spindle aberration we performed kinesin-driven microtubule gliding assays and co-sedimentation experiments. As a model motor protein we employed Ncd, a kinesin required for proper assembly of mitotic and meiotic spindles. We have demonstrated that PrP reduces binding of Ncd to microtubules leading to inhibition of Ncd-driven transport of microtubules. Noteworthy, reduction in Ncd-microtubule co-sedimentation has been observed already at low PrP to tubulin molar ratios. At the same experimental conditions PrP had no

effect on binding of Tau protein to microtubules, implying specificity of PrP action. Our results suggest that above mentioned inhibition of the transport results from PrP-induced structural changes in microtubules that hinder Ncd binding to tubulin.

By this study we want to point another plausible mechanism of PrP cytotoxicity related to the interaction with tubulin, namely impeding microtubule-dependent transport.

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P16r-46
Coenzime Q, CoQ, protects endothelial cells from ß-amyloid deleterious effects

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Neuropathological symptoms of Alzheimer's disease (AD) appears in advances stages, once neuronal damage has taken place. Nevertheless, recent studies demonstrate that in early asymptomatic stages, ß-amyloid peptide damages the cerebral microvasculature, which is nowadays believed the basis of the disease. Brain endothelial cells (BECs) are a key component of the blood brain barrier (BBB). It has been demonstrated that the ß-amyloid peptide induces necrosis and apoptosis in BECs, which reduces their proliferative and migratory abilities and creates discontinuities in the BBB, compromising the nutrients exchange. Although the mechanisms by which ß-amyloid peptide acts on ECs have not been already unraveled, it is known that its effects on cell survival and migration are preceded by an increase in reactive oxygen species (ROS) and free cytosolic Ca^{2+}, which alters the mitochondrial function. The goal of our work is to study the putative preventive effect of the lipophilic antioxidant coenzyme Q, CoQ, against ß-amyloid peptide-induced damage of human umbilical vein endothelial cells (HUVECs). The addition of ß-amyloid peptide to cultured HUVECs induced a rapid influx of extracellular Ca^{2+}, followed by Ca^{2+} release from mitochondria, due to opening the mitochondrial transition pore, but also increased O_2^- and H_2O_2 levels. In all instances, these effects were prevented when cultured HUVECs were pretreated with CoQ. However, CoQ was ineffective when administrated together with the ß-amyloid peptide. Besides, pretreatment with CoQ prevented ß-amyloid-induced HUVECs necrosis and apoptosis, restoring their ability to proliferate, migrate and form tube-like structures *in vitro*. As a whole, CoQ protects cultured HUVECs from ß-amyloid-induced damage and increases the capability of HUVECs to proliferate and heal wounds. CoQ action is cytoprotective and it is not effective once the damage is done. Therefore, CoQ could probably be a promising molecule to treat ß-amyloid-induced endothelial dysfunction in early asymptomatic AD stages, to delay the progression of the disease.

P16-47
Acute pharmacokinetics of negatively charged neuroactive steroid 3α5β pregnanolone glutamate in the rat

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Recent reports indicate that neuroactive steroids may be efficacious in a treatment of various neuropsychiatric disorders. We have designed and synthetized a novel use-dependent NMDA receptor antagonist 3α5β-pregnanolone glutamate (3α5β-P-Glu). This drug showed neuroprotective properties in our previous rodent learning studies; however, there are no pharmacokinetics data available to assess its potential as a drug for human use. Therefore, the objective of this study was to evaluate 3α5β-P-Glu pharmacokinetic properties after single dose in rat. New liquid chromatography-mass spectrometry (LC-MS) methods for selective and sensitive quantification of 3α5β-P-Glu from tissues and plasma have been developed. 3α5βP-Glu is rapidly absorbed after i.p. administration and penetrates through the blood brain barrier with a tmax of 60 min. The drug was eliminated by a first-order process and it was not accumulated in the brain or other tissues. 3α5βP-Glu was found to be preferentially distributed in the brain followed by decreasing concentrations in the kidney, liver and heart. Furthermore, 3α5βP-Glu was orally available. Taken together, our study suggests that 3α5βP-Glu have convenient pharmacokinetics properites as a neuroprotective drug in rat.

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P16-48
Neuroprotective effects of proinsulin on the loss of synaptic function and structure in a mouse model of retinitis pigmentosa

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Retinitis pigmentosa (RP) is a general term for a disparate group of retinal dystrophies characterised by progressive loss of vision leading ultimately to blindness. At present, the cellular changes that occur in the retina of patients with RP and that lead to photoreceptors death are not well characterised. Loss of funcion, apoptosis of photoreceptors and retinal disorganisation are common features in animal models of the disease. In previous work, we demonstrated that proinsulin delays vision loss and retinal degeneration in several models of RP.

The aim of the present study is to determine the molecular and cellular alterations taking place in the retina of a mouse model or RP and to identify additional potential benefits of a proinsulin-based treatment. Untreated rd10 mice or rd10 expressing human proinsulin (hPi) were used for this study. Visual function was evaluated by ERG and the optomotor test. Retinal structure and synaptic preservation were assessed by immunohistochemistry and, finally, calpain activity was measured by immunohistochemistry and western-blot.

Rd10 photoreceptors showed an early m-type calpain over activation followed by a loss of postsynaptic spikes in the bipolar cells. Expression of hPi in rd10 mice prolonged vision function, as determined by ERG and the optomotor test. Surprisingly, treatment with AAVs encoding hPi led to longer ERG response than hPi transgenic expression despite a worse cellular conservation. Hence, functional preservation may be due to a better synaptic maintenance, as determined by colocalisation of synaptic markers. The results confirm the role of calpains on retinal degeneration and provide evidence that proinsulin may play an active therapeutic role by preserving synaptic function during the neurodegenerative process.

P16-49
Ablation of *Vhl* in tyrosine hydroxylase positive cells results in abnormal sympathoadrenal development and altered acclimatization to chronic hypoxia

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The product of von Hippel-Lindau tumour suppressor gene (*VHL*) is part of a protein complex responsible for the polyubiquitylation and proteasomal degradation of the hypoxia-inducible transcription factor in the presence of oxygen. Inactivating *VHL* germline mutations in humans are associated with the appearance of different tumours, including pheochromocytoma and paraganglioma.

To generate a mouse model of paraganglioma and/or pheochromocytoma we have inactivated *Vhl* in catecholaminergic cells using the Cre recombinase under the control of the tyrosine hydroxylase (*Th*) promoter. TH-VHL animals survived well and, unexpectedly, did not generate tumours. In contrast, they showed major alterations of sympathoadrenal development, characterized by agenesis, or atrophy, of the carotid body (CB), adrenal medulla (AM) and superior cervical ganglion (SCG). CB atrophy was accompanied by a marked increase in the number and proliferative status of sustentacular (or type II) cells, which when cultured *in vitro* were unable to differentiate into viable TH^+ glomus (or type I) cells. Surprisingly, the lack of sympathoadrenal cells did not seem to compromise animals' normal growth and behavior in normoxia (21%O_2). However, they died of severe cardiopulmonary alterations few days after they were in hypoxia (10%O_2). These data indicate that CB and other sympathoadrenal organs are absolutely necessary for proper acclimatization to even relatively mild hypoxia. TH-VHL mice provide a model for studying the pathophysiology of sympathoadrenal dysfunction.

P16-50
Do structural or molecular changes in the hippocampus explain the behavioural changes observed in a mouse of foetal alcohol syndrome?

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Prenatal alcohol exposure can result in patterns of physical and mental abnormalities described as fetal alcohol spectrum disorder (FASD) in which the most severe form is fetal alcohol syndrome (FAS). The clinical features of FAS/FASD include growth restriction, with or without craniofacial abnormalities, and brain structural and functional abnormalities. The adverse outcomes of prenatal alcohol exposure are linked to exposure timing and dosage, and symptoms can vary from case to case. This makes clinical feature-based diagnosis difficult. Epigenetics is considered to play a role in the etiology of FAS/FASD, as DNA methylation, post-translational histone modifications and microRNA expression patterns have all been reported to be altered in response to alcohol exposure in animal models. Such epigenetic changes could be used as biomarkers to enable early and accurate diagnosis. In this study, a mouse model of moderate prenatal alcohol exposure, established previously, is further characterized to identify human FAS/FASD-like phenotypes. The peak maternal blood alcohol concentration measured is 96 mg/dl, and the exposure period is from gestational day 0.5–8.5. This model mimics chronic, moderate drinking behavior during the first 3 weeks of pregnancy in humans. Magnetic resonance imaging-based structural analyses of the brain revealed a significant reduction of relative hippocampal and caudate putamen volume compared to whole brain volume in the ethanol-exposed group compared to controls. This is reminiscent of changes seen in human FAS/FASD. Moreover, significant behavioural changes are also observed in the treatment group across three independent Cohorts. Microarray analyses of various brain regions revealed a number of differentially expressed genes (>1.5 fold change) in the treatment group compared to controls. qPCR based Genome-wide miRNA profiling demonstrated that a few of miRNAs were dysregulated in the hippocampus associated with the prenatal alcohol exposure. Currently, mRNA-miRNA modules and any related epigenetic control are under identification both *in silico* and *ex vivo*. The possible relationships between the ethanol-induced molecular and phenotypic changes and their contribution to the etiology of FAS will be discussed.

P16-51
Evidences of ATP production in myelin sheath: a new trophic role of myelin sheath

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CNS is an organ with high energy demand, even though its mitochondrial density is low. It was suggested that part of the energy may be supplied by the glia. Our attention has focused on myelin sheath because there is growing evidence that it also has a neurotrophic role. In fact, in demyelinising diseases like Multiple Sclerosis, the loss of myelin causes an axonal necrosis, cause of the neurological disability.

Aiming at contributing to the understanding of the causes of the axonal degeneration consequent to myelin loss, our principal objective was to demonstrate that the whole glucose catabolism is functional in myelin, to carry out the oxidative phosphorylation, for ATP supply to the axon.

Experiments were conducted on isolated myelin vesicles (IMV). Both an imaging and a biochemical approach was utilized. Confocal microscopy as well as oxymetric, fluorimetric, luminometric and semiquantitative western blotting (WB) analyses were performed to identified the respiratory chain in IMV and to observe oxygen consumption, ATP synthesis and ETC activity, respectively. The IMV quality was tested by WB and electron microscopy.

We observed that IMV: (i) consumes oxygen when energized with NADH and Succinate and other unconventional respirating substrate; (ii) displays a proton gradient across its surface; (iii) contains F_oF_1-ATP synthase and the four ETC, which are catalytically active and sensitive to the common inhibitors [1,2]; (iv) contains all of the glycolytic and the Krebs Cycle enzymes. The extent of IMV mitochondrial contamination was assessed with specific Ab by WB, and it was found that ANT, TIM and TOM and Adenylate Kinase Isoform 3 (mitochondrial) activity were absent.

Data suggest that myelin is a site of glucose combustion and that it is catalytically active for aerobic ATP production. This basic study will shed light on the etiopathogenesis of many demyelinising diseases [3].

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P16-52
Investigation of the immunogenicity of amyloid beta oligomers

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Many studies concerning therapeutic and prophylactic approaches for Alzheimer's disease (AD) are based on the anti-amyloid immunotherapy. It has been suggested that either active immunization with amyloid beta (Aβ) protein or passive immunization with anti-Aβ antibodies might help to prevent or reduce the symptoms of AD. However, the knowledge on the mechanisms of Aβ-induced immune response is rather limited. Our recent investigations show that the cytotoxicity of Aβ oligomers greatly depends on their size: small oligomers were cytotoxic in primary cultures of rat cerebellar granule cells whereas the bigger aggregates did not cause detectable neuronal death [1]. In the current study we evaluated the influence of the shape and size of Aβ-oligomers on their immunogenicity. For this purpose we investigated the capability of various Aβ1-42 cytotoxic oligomers to induce antibody response in immunized mice. The analysis of titres and isotypes of serum antibodies revealed that the small Aβ cytotoxic oligomers (1–2 nm in diameter) are highly immunogenic. In contrast, larger Aβ oligomers, Aβ fibrils and monomers did not induce a detectable IgG response in immunized mice. Epitope mapping demonstrated that the main immunodominant region of the cytotoxic Aβ1-42 oligomer is located at its N-terminus. The results of the current study might be important for the development of Aβ-based vaccination and immunotherapy strategies.

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P16-53
Anosmin-1 domains play different roles in the binding to FGFR1 and the function of the protein

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The protein anosmin-1 coded by the *KAL1* gene, is responsible for the sex-linked form of Kallmann syndrome (KS). This protein has a role in adhesion, migration and differentiation of different cell types in the CNS. The best known mechanism of action of anosmin-1 is through the interaction with and modulation of FGFR1 activity.

By means of GST pull-down we have identified the domains of anosmin-1 involved in the interaction with FGFR1 and studied the effect on this interaction of several missense mutations found in KS patients. We have also studied the effect of these missense mutations and different nonsense mutations found in KS patients, on the chemo-attraction exerted by anosmin-1 via FGFR1 on rat neuronal precursors. We have described the interaction of anosmin-1 with fibronectin, laminin and anosmin-1, defined the interacting domains of anosmin-1 and the effect of some KS missense mutations in the interactions. We are studying the effect of anosmin-1 on cortical oligodendrocyte precursor cell (OPCs) migration and the signalling pathway activated through FGFR1 by this protein on OPCs from rat, mouse and the cell line GN11 derived from GnRH neurons. Surprisingly, some of the pathways activated by anosmin-1 seem to be triggered by a mechanism independent of FGFR1.

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P16-54
Kainate receptor-mediated modulation of glutamatergic transmission at the lateral amygdala involves protein kinase A

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Kainate receptors (KARs) have been described as modulators of synaptic transmission at different synapses. However, this role of KARs has not been well characterized in the amygdala. We have explored the effect of kainate receptor activation at the synapse established between fibers originating at medial geniculate nucleus and the principal cells in the lateral amygdala.

By using whole-cell patch-clamp techniques, we have observed an inhibition of evoked excitatory postsynaptic currents (eEPSCs) amplitude (67 ± 5%, n = 12) after a brief application of 1 μM kainate (KA). Paired-pulse recordings showed a clear facilitation (2.2 ± 0.1%, n = 6) that was increased after KA application (3.8 ± 0.2%, n = 6). The loading of postsynaptic cells with BAPTA (20 mM), did not prevented the KA effect (depression of 59 ± 15%, n = 4). This effect of KA was prevented when the slices were treated with the protein kinase A inhibitors, H-89 (2 μM, 17 ± 4%, n = 6) or Rp-Br-cAMP (100 μM, 8 ± 5%, n = 6). Taken together our results indicate that KARs present at this synapse are presynaptic and their activation mediate the inhibition of glutamate release through a mechanism that involves the activation of protein kinase A.

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P16-55
Endocytosis of TLR4 via caveolae is necessary for ethanol-mediated inflammation in astroglial cells

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We demonstrated that ethanol, by activating TLR4 signaling in glial cells, is able to induce proinflammatory mediators in the brain causing neuroinflammation and brain damage. Our results further suggest that ethanol acts as agonist of TLR4, since both LPS (ligand of TLR4) and ethanol promote the recruitment of TLR4 and signaling molecules into lipid rafts microdomains.

However, how TLR4 is internalized and how this event influences TLR4 signaling remain elusive.

The aim of this study was to elucidate the endocytic pathway of TLR4 in response to LPS or ethanol in primary astroglial cells. For this, we develop a recombinant adenovirus expressing the fusion protein TLR4:GFP achieving infection efficiencies of ~65% according to flow citometry. After infection, we studied the expression of TLR4:GFP by RT-PCR, western blot and immunofluorescence and its function evaluated by measuring cytokines. Internalization of TLR4 was assessed upon LPS or ethanol stimulation. To determine the endocytic pathway, we use *dynasore*, a non-competitive inhibitor of dynamin GTPase activity that blocks dynamin-dependent endocytosis, and *saponin* which inhibits the caveolae-mediated uptake. We found that endocytosis of TLR4 is dependent on dynamin and mainly occurs via lipid rafts. In addition, our results show that endocytosis is necessary for receptor signaling. Using confocal microscopy, we demonstrate that TLR4 is internalized via caveolae pathway upon LPS or ethanol treatment, as observed by the colocalizacion of TLR4 with caveolin-1 or Ctx-B. In conclusion, we provide evidence for the first time that ethanol triggers the endocytosis of TLR4 via caveolae and that this event is crucial for the initiation of the receptor signaling (Supported by SAF-2009-07503).

P16-56
Extramitochondrial oxidative phosphorilation in retinal rod outer segments in different animal models

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The rod Outer Segment (OS) disk, devoid of mitochondria, is specialized in phototransduction, a process requiring a consistent chemical energy supply. Our previous results from proteomic, classical biochemical metabolic and imaging studies of purified bovine retinal OS disks, showed the functional presence of mitochondrial FoF1-ATP synthase, and respiratory chain complexes I to IV in OS (1). Here the expression of oxidative phosphorylation (OXPHOS) proteins in disks is assessed in three animal models: bovine, mouse and zebrafish (*Danio rerio*). Atomic Force Microscopy (AFM) analysis on disks and Transmission Electron Microscopy (TEM) on retinal sections confirm the presence of ATP synthase on bovine disks. Immunocitochemistry analysis on mouse retina sections demonstrated the activity of complexes I to IV in OS.

Retinal structure of zebrafish, a typical experimental vertebrate model system for eye studies, displays the typical vertebrate pattern, and by 3 days postfertilization photoreceptors are differentiated (2). We report colocalization of zpr3 (rod opsin) with OXPHOS proteins in adult and embryo zebrafish at different developmental stages (4, 8 and 12 days). Data are discussed in terms of aerobic energy supply for phototransduction, following the hypotheses of an intraflagellar transport (IFT) delivering the OXPHOS proteins to the OS.

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P16-57
Proteostasis of SMN and SMNΔ7 proteins implicated in spinal muscular atrophy

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Spinal muscular atrophy (SMA) characterized by the loss of lower motor neurons and atrophy of muscle is the leading genetic cause of infant mortality. There are two SMN genes in humans, SMN1 (telomeric) and SMN2 (centromeric), in chromosome 5q13 SMN1 gene encodes the full-length protein (SMN) and the SMN2 gene preferentially generates an exon 7 deleted protein (SMNΔ7) by alternative splicing due to a C to T transition. Previous reports have shown that both SMN (half-life 4.3 hour) and SMNΔ7 are unstable proteins by radioactive pulse-chase experiments, being degraded by the ubiquitin-proteasome pathway, having SMNΔ7 a shorter half-life (2.2 hour) than SMN, indicating that protein degradation is crucial in SMN proteostasis. In contrast, we have found that endogenous (HeLa) and transfected SMN proteins are rather stable proteins as judged by treatment of cells with protein synthesis inhibitors, with a half-life longer than 24 hour. Our results are in agreement with recently published data of the determination of SMN turn-over under steady-state conditions by SILAC/MS quantitative proteomics. Transfection of HeLa cells with a SMNΔ7 protein construct and treatment with protein synthesis inhibitors has allowed us to show that this protein has much shorter half-life than the SMN protein and its degradation is prevented by proteasome inhibitors. These results clearly indicate that the instability of SMNΔ7 is due to its increased degradation rate (pulse-labeled and protein synthesis inhibition studies) and its steady state concentrations in the cell are mainly determined by its rate of synthesis. The newly synthesized SMN protein has also a short–half life (pulse-chase labeled studies), but its steady-state concentration in the cell is mainly determined by the long half-life of the SMN protein that escape the early post-translational quality control (studies with protein synthesis inhibitors and SILAC/MS studies), The stabilization of SMN is likely due to SMN post-translational modifications and protein-protein interactions that play a major role in SMN turnover and proteostasis.

P16-58
Influence of genotype on hippocampal expression of lipogenic genes in a transgenic model of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegeneration characterized by the presence of senile plaques and neurofibrillar tangles in the brain, leading to a progressive neuronal loss in cortical and hippocampal areas, and a dramatic decrease of cognitive functions.

Apart from adipose tissue, the brain is the organ containing the largest amount of lipids. The lipids in the central nervous system contain large proportions of long chain polyunsaturated fatty acids (LCPUFA), especially AA (arachidonic acid, 20:4n-6) and DHA (docosahexanoic acid, 22:6n-3).In the last decades, an increasing interest has focused on the putative neuroprotection

by polyunsaturated fatty acids (PUFA). It is well-known that DHA is required for brain and retina development and has been implicated in excitable membrane function, synaptogenesis and neuroprotection. Moreover, the levels of DHA and AA are reduced in AD brains as compared to aged patients, reflecting a deficiency in the ability to desaturate and elongate the precursors 18:2n-6 and 18:3n-3.Though the molecular mechanisms underlying neuroprotection by PUFA are largely unknown, the potential neuroprotective effects of n-3 PUFA could be elicited by a sort of compensatory corrections of lipid defects in neural membranes.

In this study, we have aimed at determining the expression levels of central genes involved in the LCPUFA biosynthetic pathways, in the hippocampus of wild type and APP695sswe/PS1 mice, a transgenic model of familial Alzheimer's disease. Our data indicates that both the genotype and the dietary regimes strongly affect the relative expression of LCPUFA lipogenic enzymes in hippocampal nerve cells. A significant interaction between genotype and dietary conditions could also be observed.

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P16-59
Parkinson's disease: evaluating the effects of possible anti-parkinsonian compounds on alpha-synuclein aggregation and neurotoxicity

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The aggregation of many proteins and polypeptide chains is the hallmark of many diseases called collectively as amyloidosis. Parkinson's disease (PD) is a chronic disorder characterized by the formation of intraneuronal inclusions called Lewy bodies mainly composed of a-synuclein (AS), a natively-unfolded protein. Up to now, the only available treatment administered to PD patients is L-dopa, the precursor of dopamine.

Selegiline (Sel) is a monoamino oxidase-B inhibitor with neuroprotective effects. It has been administered to PD patients either as monotherapy or in combination with L-dopa. Our group has evaluated the effect of Sel in the *in vitro* aggregation of wt and A30P a-syn. We could not map any specific interaction of Sel with monomeric AS but our data showed that Sel was able to delay AS fibril formation leading to the formation of an aberrant aggregate of AS, which showed to be non-toxic to domaminergic neurons in culture. Our data showed that Sel acts specifically in the nucleation phase of AS aggregation. Taken together these data suggest that administration of Sel to PD patients abolished the formation of toxic aggregates in route to fibril formation.

Edaravone (ED) has anti-oxidant properties and has neuroprotective effects against 6-hidroxidopamine induced neurodegeneration. We also tested the effect of ED on AS aggregation. Our data shows that ED seems to interact with WT AS, as observed in NMR experiments, modulating its aggregation and is neuroprotective against the toxicity of a-syn oligomers.

These data will be discussed in the light of possible therapies against PD.

P16-60
Molecular control of stem cell activity in the adult brain

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Neural stem cells (NSCs) generate new neurons throughout life in two distinct areas of the mammalian brain, the subventricular zone lining the lateral ventricles and the hippocampal dentate gyrus. Adult neurogenesis has been implicated in tissue homeostasis, physiologic brain function, and is also associated with a number of neuro-psychiatric diseases, such as cognitive aging and depression. Understanding the mechanisms underlying adult neurogenesis represents a prerequisite for future therapeutic targeting of adult NSCs for endogenous brain repair. Here I will summarize our efforts to understand the cellular and molecular mechanisms underlying distinct steps in the developmental course of adult neurogenesis, from the dividing NSC to the integrating neuron. I will present recent data where we used unbiased gene expression profiling of adult NSCs and their neuronal progeny directly isolated from the adult hippocampus. With this approach we found that adult NSCs appear to be in a distinct metabolic state compared to their differentiated progeny and other dividing neural cells. Adult NSCs are in a lipogenic state and have high levels of de novo lipid synthesis, a metabolic state that is similar to many cancer cells. Using pharmacological and conditional genetic deletion of key lipogenic enzymes, we show that de novo lipogenesis is required for proper stem cell proliferation in the adult brain. Thus, our data identify a previously unnoticed and fundamentally important role for lipid metabolism in adult neurogenesis, that may represent a novel pharmacological target for therapeutic manipulation of neurogenesis in neuro-psychiatric diseases.

P16-61
The axonogenic effect promoted by oleic acid during early postnatal development is related with the expession of FABPs proteins in SVZ and striatal cells

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Oleic acid is synthesized by astrocytes in response to albumin and promotes neuronal differentiation together with the increase of GAP43 and MAP2 expression (markers of axonal and dendritic differentiation, respectively). We have studied the intracellular transport of oleic acid in neurons. We have found that 'fatty acid binding proteins' (FABPs) participate in the signal transduction by which oleic acid promotes neuronal differentiation. We have studied the possible changes in FABP-3, -5, -7 localization into neurons and the changes in their expression. In order to get insight unto the role played by these proteins in the mechanism triggered by oleic acid we silenced FABPs expression by siRNA and we analyzed the change in GAP43 and MAP2 expression caused by the presence of oleic acid.

The levels of FABP3 increased by oleic acid and this protein colocalized in the growth base of neurites with GAP43. In addition, FABP5 is essential for neuronal survival because blocking the expresion of this protein induced apoptotic death. Likewise, FABP7 expression decreased in the presence of oleic acid and this may be associated with neurite differentiation, showed by MAP2 expression.

We used organotypic cultures to study the effect of oleic acid on SVZ and striatal neurons. We analyzed the evolution of molecular markers such as GAP43, MAP2 and DCX together with FABPs and albumin in the presence of albumin and albu-

min-oleic acid complex. We also studied the effect of blocking FABPs expression under these conditions. Our results show that FABP3 is induced in differentiated neurons of striatum; FABP5 is essential to cellular survival and it is mainly localized in SVZ areas; FABP7 which is expressed in radial glial cells can be related with the migration of oleic acid targeted cells.

P16-62
17-betaestradiol increases ethanol induced disruption of blood–brain barrier; responsibility of inducible nitric oxide synthase

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The blood–brain barrier (BBB) that saves the brain from toxic substances is a functional unit formed by endothelial cells. It is mainly composed by tight junction (TJ) proteins that exist between endothelial cells. Estrogen is an important hormone to regulate BBB permeability which protects BBB before menopause, but with aging may enhance BBB permeability. In addition, nitric oxide also modulates BBB permeability. Alcohol also impairs the integrity of the BBB with oxidants and inflammatory mediators such as iNOS. Using the *in vitro* BBB model HUVEC/C6, which consists of cocultures of human umbilical vein endothelial-like cells (HUVEC) and rat glioma cells (C6), the effect of beta estrodiol on ethanol induced BBB disruption and change/or increase of iNOS activity were evaluated. Inducibility and functionality of tightness (indication of BBB integrity) were investigated by transendothelial electrical resistance (TEER). iNOS activity was also investigated by immunstaining. As an indicator of invitro BBB formation, presence and inducibility of TJ proteins and increase of TEER measurement were proven for HUVEC layers. Long term treatment of β-estradiol alone decreased TEER, amount of occludin and claudin-1. This adverse effect of β-estradiol got worse with addition of ethanol and increased iNOS activities. After ethanol treatment, short term β-estradiol treatment decreased claudin-1 and iNOS but had no effect on occludin amount. We determine that 17-beta estradiol used alone or in combination with ethanol did not showt a healing effect on the *in vitro* BBB, established with estrogen receptor alpha lack HUVEC cell line (this model may mimic menapausal period in woman). Long-term 17-beta estradiol treatment before ethanol further enhanced ethanol's destructive effects on BBB. Thus it can be said that estrogen replacement therapy during menopause causes BBB damage and constitutes the risk of cerebrovascular diseases. Our study is unique in this regard and shed light on further studies.

P16-63
Extramithochondrial production of ATP in myelin sheat: correlation among demyelination and axonal degeneration in multiple sclerosis

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Multiple Sclerosis (MS) is an inflammatory-mediated demyelinating disease, which affects the human central nervous system (CNS). During the disease, the myelin sheath loss causes a slowdown of the nerve impulses between the CNS and the rest of the body, and a progressive axons degeneration. This axonal transection is the major determinant of irreversible neurological disability in MS patients and it suggests that myelin sheath is not only an axons insulator, but a structure with a own trophic role. Recently, it was hypothesized that axonal degeneration depends on an energy imbalance. Interestingly, we found that electron transport chain and ATP synthase are present in vesicles of isolated myelin, and that they produce ATP by oxygen consumption [1]. Consequently, the demyelination, occurring during MS, may cause an ATP depletion which leads the axon loss of functionality and necrosis. By both biochemical and imaging techniques, we observed that the presence and the activity of the respiratory chain in myelin sheath decrease in the MS plaques and this impairment is proportional with the seriousness of lesions. Moreover, we hypothesize that this respiratory chain dysfunction could depend by the production of reactive oxygen species, that, damaging the membrane, could cause further loss of myelin sheath. This results may give a new input to understand the demyelination process and its correlation with the axonal degeneration.

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P16-64
Role of SNAP-25 phosphorylation by protein kinase A in SNARE complex formation

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Exocytosis is one of the most fundamental cellular events. The basic mechanism of the final step, membrane fusion, is mediated by the formation of SNARE complex, which consist of three proteins, syntaxin, SNAP-25 (synaptosome-associated protein of 25 kDa) and VAMP (vesicle-associated membrane protein). Protein phosphorylation represents a regulatory mechanism of exocytosis. It has been reported that SNAP-25 is phosphorylated by protein kinase A (PKA). However, the role of SNAP-25 phosphorylation by PKA in exocytosis, particulary in the formation of SNARE complex remains still elusive. In the present study, we identified Ser28, Thr29, Thr138 and Ser187 of SNAP-25 as possible phospho-residues by PKA using site-directed mutagenesis. The formation of sodium dodecyl sulfate (SDS)-resistant complex was assayed using all of three purified recombinant SNARE proteins; the complex formation was decreased when wild-type SNAP-25 was phosphorylated by PKA in advance, whereas PKA treatment of T138A mutant of SNAP-25 showed little effect, indicating that phosphorylation of T138 results in the decreased complex formation. The level of SDS-resistant SNARE complex in PC12 cells was also reduced after forskolin treatment. The level of both syntaxin and VAMP co-precipitated in anti-SNAP-25 immunocomplex isolated from PC12 cells were also decreased after forskolin treatment. These results suggest that phosphorylation of SNAP-25 by PKA exhibits inhibitory role in SNARE complex formation.

P16-65
Lipid microdomain-associated β-hexosaminidase and β-galactosidase activities increase in brain cortex of Alzheimer's disease mouse model TgCRND8

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Previously we demonstrated that lysosomal glycohydrolases β-hexosaminidase (Hex) and β-galactosidase (Gal) are up-regulated in Alzheimer's disease (AD) patient fibroblasts [1]. Here we performed lipid microdomain purification from brain cortex of both pre-symptomatic (3 months old) and symptomatic (6 months old) AD mouse model TgCRND8 and assayed glycohydrolase Hex and Gal activities in flotillin-2 positive fractions [2]. In this manner we report direct evidence of the association of glycohydrolases Hex and Gal on brain cortex lipid microdomains and show an increase of their activity in both pre-symptomatic and symptomatic mice with respect to the age matched control mice. Interestingly, lysosomal activity of Gal was also significantly increased in symptomatic and pre-symptomatic brain cortex extracts with respect to the controls. Finally, we carried out immunoprecipitation of purified lipid microdomains using specific antibody against pre- and post-synaptic markers synaptophysin and PSD-95, respectively and demonstrated the association of Hex and Gal also with post-synaptic vesicles. On the basis of these results we could hypothesize that: (i) changes of glycosphingolipid structures at the plasma membrane domain level may occur directly *in situ* to define the curvature properties of specific areas of the plasma membrane, such as synapses; (ii) the early increase of lipid microdomain-associated Hex and Gal activities, highlighted in the brain cortex of TgCRND8 mouse, may be caused by an alteration of lysosome-to plasma membrane transport pathway that regulates cellular clearance. The identification of early defective pathways is fundamental to obtain reliable biological markers to predict or confirm AD in order to carry out early therapeutic protocols [3].

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P16-66
TFEB overexpression promotes the translocation of lysosomal glycohydrolases β-hexosaminidase and β-galactosidase to cell surface lipid microdomains

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The lysosomes are membrane-enclosed organelles, containing acid hydrolase enzymes, that mediate a variety of physiological processes, such as macromolecule degradation, lipid homeostasis, energy metabolism and pathogen defence. Recently, it has been demonstrated that adaptive response of the lysosomes to physiologic signals is related to the activity of the transcription factor EB (TFEB). TFEB overexpression induces autophagy, lysosome biogenesis, up-regulation of lysosomal genes expression, and leads to clearance of storage material in several lysosomal storage disorder cell models by promoting lysosomal exocytosis [1], which is in turn responsible for the secretion of lysosomal content in extracellular environment and repair of plasma membrane (PM). In a recent work, in which the association of fully processed glycohydrolases β-hexosaminidase (Hex) and β-galactosidase (Gal) to PM lipid microdomains has been provided [2], we speculated on the existence of a lysosome-to-PM transport pathway mediating the translocation of lysosomal membrane-associated enzymes to the cell surface [2,3,4]. To test this hypothesis we transfected Hek 293 cells by using a TFEB expressing vector. Stable TFEB overexpression significantly increased lysosomal Hex and Gal activities and triggered their recruitment on cell surface lipid microdomains. Taken together these observations suggest that up-regulation of the lysosomal system due to TFEB over-expression is mirrored by a lysosomal glycohydrolases recruitment to the PM, where they may be involved in glycosphingolipids oligosaccharide modification processes regulating cell-to-cell and/or cell environment interactions in both physiological and pathological conditions.

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P16-67
Investigating the role of the cellular prion protein in Alzheimer's disease

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The mechanism of prion related neurodegeneration and the physiologic role of cellular prion protein (PrP^C) are still ill-defined. Use of animal and cell models has underscored a number of putative functions, suggesting that PrP^C serves in cell adhesion, migration, proliferation and differentiation, possibly by interacting with extracellular partners, or by taking part in multi-component signaling complexes at the cell surface. In this respect, we have demonstrated that PrP^C influences local Ca^{2+} movements in neurons. Recently, some reports have also shown that PrP^C acts as a high-affinity receptor for the amyloid-β (Aβ) peptide, a fragment of the amyloid precursor protein implicated in Alzheimer's disease (AD), and that PrP-Aβ interactions may be fundamental for AD-related impairment of synaptic plasticity. Given that synaptic plasticity is closely related to Ca^{2+} homeostasis, we have adopted the aequorin strategy to investigate whether treatment of primary cerebellar granule neurons (CGN)–expressing or not PrP^C–with monomeric or oligomeric Aβ (1-40) and (1-42) peptides, deranges Ca^{2+} metabolism in a PrP-dependent manner. Specifically, we have analysed Ca^{2+} variations in plasma membrane domains following Ca^{2+} entry through both store-operated channels and NMDA and other glutamate receptors.

P16-68
Biochemical characterization of novel Taxus lignans as effective mediators for GSK-3β and the phosphorylation of functional brain proteins by the kinase

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Object: Several novel Taxus lignans identified from Taxus yunnanensis have been characterized as potent anti-inflammatory and neuroprotective agents. Using three brain proteins [MBP, TP (tau protein) and CRMP-2] as effective protein substrates for GSK-3β *in vitro*, three Taxus lignans [A, C and tanegool (E)] were selected as effective mediators for the kinase. Therefore, the present study was done to characterize their novel effects on the GSK-3β-mediated phosphorylation of these brain proteins involved in the neuronal functions.

Results and Discussion: We found that (i) three Taxus lignans as well as EGCG highly stimulated the GSK-3β-mediated phosphorylation of MBP and TP, but inhibited the phosphorylation of CRMP-2 (an acidic protein) by the kinase; (ii) E, but not EGCG, highly stimulated autophosphorylation of GSK-3β through its induction of Tyr-phosphorylation on the kinase; and (iii) E induced the novel potent phosphorylation sites for GSK-3β on MBP and TP. Our results suggest that (i) E acts as an effective activator for autophosphorylation of GSK-3b and for the GSK-3b-mediated high phosphorylation of MBP and TP by the different activation of EGCG; (ii) the E-induced inhibition of the GSK-3β-mediated CRMP-2 phosphorylation may be involved in the promotion of microtubule polymerization at the cellular level; (iii) this lignan may be a potent suppressor for the GSK-3β-mediated CRMP-2 phosphorylation and an abnormal aggregation of phosphorylated TP with its associated proteins in the initial stage of neurodegrative diseases.

P16-69
Sleep is regulated by prostaglandin D2 selectively produced in the meninges by lipocalin-type prostaglandin D synthase

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Prostaglandin D2 (PGD2) is the most abundant prostaglandin produced in the brain. Nano-molar injections of PGD2 in rat brains demonstrated its dose and time dependent somnogenic activity, while PGD2-induced sleep was indistinguishable from physiological sleep. PGD2 can be produced by two distinct types of PGD2 synthase (PGDS), lipocalin-type PGDS (LPGDS) and hematopoietic PGDS (HPGDS), but only LPGDS is related to sleep. Three potential sites for PGD2 synthesis by LPGDS have been identified in the brain, i.e. oligodendrocytes (OD), epithelial cells of the choroid plexus (CP) and arachnoid trabecular cells of the leptomeninges (LM). We have embarked on a program to identify the site of synthesis of somnogenic PGD2 and generated a transgenic mouse line with the LPGDS gene amenable to conditional deletion using Cre recombinase. To identify which tissue is responsible for the production of somnogenic PGD2, we engineered animals lacking the expression of LPGDS specifically in:

1 the OD by cross-breeding flox-LPGDS mice with Nestin-Cre mice, inducing a complete KO of LPGDS in the neural but not in the leptomeningeal cells (OD-LPGDS KO mice).

2 the CP by injecting adeno-associated virus (AAV), serotype 5, expressing Cre recombinase (AAV5-Cre) in the lateral third ventricle (CP-LPGDS KO mice).

3 the LM by injecting AAV, serotype 8, expressing Cre recombinase (AAV8-Cre) into the ventricle of new born mice (LM-LPGDS KO mice).

We recorded electroencephalogram, electromyogram and locomotor activity to measure sleep of 10 weeks old animals with a specific knockdown of LPGDS in one of the three target tissues. By using selenium tetrachloride, a specific PGDS inhibitor, we demonstrated that sleep was inhibited in OD-LPGDS and CP-LPGDS KO mice, but not in the mice lacking LPGDS in the LM.

We concluded that leptomeningeal cells, but not OD or CP, are the souece of somnoqenic PGD2.

P16-70
Direct interaction of phospholipase C-related but catalytically inactive protein with syntaxin 1 and SNAP-25 through the C2 domain

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PRIP (Phospholipase C-related but catalytically inactive protein) was first identified as a novel Ins(1,4,5)P_3 binding protein. We have recently found that the secretion of various peptide hormones from multiple organs were up-regulated in PRIP knockout mice, indicating that PRIP regulates the exocytic event in a negative manner. To explore the molecular mechanism underlying the inhibitory role of PRIP in exocytosis, we examined the role of the C2 domain of PRIP (PRIP-C2). We detected no trace of lipid binding of PRIP-C2 in a lipid overlay assay, although C2 domains are well-recognized as a Ca^{2+}-dependent phospholipid-binding domain. On the other hand, regulatory proteins such as synaptotagmin and rabphilin, bearing C2 domain, regulates exocytosis *via* binding to SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins. Thus, we investigated the interaction of PRIP with SNARE proteins using pull-down assay and proteo-liposome floatation assay, and found that PRIP interacts with the components of SNARE complex including syntaxin 1 and SNAP-25 (synaptosomal-associated protein of 25 kDa) only through the C2 domain in a Ca^{2+}-dependent manner. To examine the role of the binding of PRIP-C2 with SNARE proteins, we tested the effect of PRIP on the SNARE complex formation. PRIP inhibited the formation of stable SDS-resistant SNARE complexes in a dose-dependent and a Ca^{2+}-dependent manner. The results suggest that PRIP-C2 is implicated in PRIP-mediated inhibition of exocytosis through the direct interaction of the C2 domain of PRIP with syntaxin 1 and SNAP-25.

P16-71
Involvement of mTOR in diacylglycerol kinase β (DGKβ)-induced neurite blanching and spine formation

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Background and purpose: Diacylglycerol kinase (DGK) converts diacylglycerol (DG) to phosphatidic acid (PA), resulting in attenuation of protein kinase C (PKC) activity by reducing DG. PA is also an important lipid messenger to activate other enzymes including mammalian target of rapamycin (mTOR). We

have recently reported that overexpression of DGKβ, which is enriched in neurons among ten mammalian subtypes, induces neurite branching and spine formation. However, molecular mechanism of DGKβ-mediated spine formation and branching is still unknown. On one hand, there are some reports showing that mTOR are involved in the spine formation. Therefore, we investigate whether mTOR is involved in The DGKβ-mediated spine formation and neurite branching.

Methods: GFP-DGKβ or GFP alone was overexpressed in SH-SY5Y by lipofection or in mouse primary cultured cortex or hippocampus neurons by infection. Twenty four hours after lipofection on infection, the cells were treated with mTOR inhibitors for additional 24 hour. Finally, number of branches and spines were counted can compared with control using confocal laser scanning fluorescent microscope.

Results and Discussion: Overexpression of GFP-DGKβ in SH-SY5Y cells induced branching but this was inhibited by specific inhibitor of mTOR, Ku0063994 and PP242, indicating that mTOR is involved in the DGKβ-induced branching. Generally, mTOR functions via two major distinct protein complexes, TORC1 and TORC2. To identify which complex is involved in the DGKβ-induced branching, effect of rappamycin was investigated because only TORC1 is sensitive to the drug. Rapamycin inhibited the DGKβ-induced branching in SH-SY5Y and in primary cultured cortex neurons. Similarly, DGKβ-induced spine formation in hippocampus neurons was inhibited by rapamycin. These results indicate that DGKβ activates mTOR in TORC1 through PA production, resulting in neurite blanching and spine formation.

P16-72
The 5,10-methylenetetrahydrofolate reductase 1298A>C polymorphism genotype influences the association of cortisol serum levels to Alzheimer's type dementia

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Introduction: High levels of cortisol and mutations in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene have been proposed as risk factors for Alzheimer's disease. However, the interaction between these risk factors in association to Alzheimer's disease has not been investigated and was the aim of the present study.

Methods: The subjects (> 50 years of age) were composed of 27 individuals diagnosed with Alzheimer's type Dementia (ATD) (test group) and 25 healthy individuals matched for age and gender (control group). The study was approved by the local Ethical Committee. Blood samples were collected and serum concentrations of cortisol were determined. MTHFR 1298A > C polymorphism was screened by PCR-RFLP.

Results: Serum levels of cortisol were higher in patients than in controls (17.2 ± 0.7 μg/dl versus 15.2 ± 0.6 μg/dl, $p < 0.03$). In men, the frequency of individuals carrying at least one MTHFR 1298C allele was higher in patients (69%) than in controls (14%, $p < 0.02$). Similarly, univariable logistic regression analysis showed a positive association of cortisol (OR = 1.19, $p < 0.03$) to ATD while a positive association of the MTHFR 1298AC or CC genotypes (OR = 13.5, $p < 0.02$) to ATD was found in men. After stratifying individuals taken into account the MTHFR 1298A > C polymorphism, an association between cortisol (OR = 1.72, $p < 0.002$) and ATD was still observed for individuals with the MTHFR 1298AA but not with the AC or CC genotypes, suggesting an interaction between cortisol and the MTHFR 1298A > C mutation in association to ATD, which was confirmed by multivariable logistic regression analysis (interaction coefficient = -0.50, $p < 0.05$).

Conclusion: The association of cortisol to ATD seems to be dependent on the MTHFR 1298A > C polymorphism genotype.

P16r-73
Pikachurin and its interacting glycoprotein, dystroglycan, are located in the outer plexiform layer of the human retina

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Dystroglycan (DG), a key component of the dystrophin-glycoprotein complex, is composed of an extracellular α-DG subunit and a transmembrane β-DG subunit, and provides a physical nexus between components of the extracellular matrix and the cytoskeleton of CNS and muscle cells. Pikachurin, a retinal protein essential for the formation of ribbon synaptic structures between photoreceptors and bipolar cells, has been recently reported as an extracellular ligand for α-DG in the mouse. Also, it is known that proper O-glycosylation of the α-DG subunit is critical for the formation of the DG-pikachurin complex and thus for proper visual function. One of the main enzymes responsible for this process is the so-called protein O-mannosyltransferase 1 (POMT1), which adds the first residue of the O-mannosylglycan chains attached to the α-DG molecule.

In the present study we have analyzed by immunohistochemical methods the distribution of α- and β-DG together with that of POMT1 and pikachurin in the human retina. We have observed that both DG subunits are present: (i) in the outer plexiform layer (OPL), where they concentrate at photoreceptor axon terminals (cone pedicles and rod spherules), (ii) in the inner limiting membrane (ILM), which is secreted by Müller glial cells and separates the retina from the vitreous, and (iii) around retinal blood vessels. Consistently, POMT1 is expressed mainly by photoreceptors and Müller cells, among other retinal neuronal types. In a similar fashion, we have found that pikachurin is immunolocalized in the OPL, and especially at cone pedicles. These results are indicative that POMT1 could exert a role in α-DG O-glycosylation in the adult human retina, which in turn should be crucial for its proper interaction with pikachurin in the OPL and hence for a correct structure and function of ribbon synapses established between photoreceptors and their postsynaptic neurons.

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P16-74
Inducing neural progenitor cells proliferation by the diterpene 3,12-di-O-acetyl-8-O-tigloylingol from *Euphorbia lactea*

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Generation of new neurons form neural stem cells is induced in the central nervous system in response to different types of injuries. Within the injured areas, neural stem cells are activated to produce neural progenitor cells (NPCs) which proliferate and differentiate into neurons and/or glial cells in an attempt to repair the damaged tissue. Understanding the mechanisms involved in the activation of neural stem cells in injured areas would facilitate the development of strategies aimed to repair brain lesions by either facilitating the activation of endogenous neural stem cells in the injured areas or by using transplants of NPCs previously expanded *in vitro*.

Based on previous results obtained by our research group and on structural similarities with non-tumor promoter diterpenes such as prostratine, compounds with lathyrane skeleton have been proposed as molecules agonist of proteins kinase C (PKC).

Therefore, we aim to evaluate the mechanisms of action of a lathyrane-type diterpene able to promote NPC proliferation. Cultured mouse subventricular zone NPCs treated with 10 μM of 3,12-di-*O*-acetyl-8-*O*-tigloylingol (ELAF) isolated from the plant *Euphorbia lactea* showed higher proliferative capacity than control cultures when stimulated with either EGF, bFGF or a combination of both.

In the presence of PKC inhibitor G06850, NPC proliferation was not stimulated by treatment with ELAF, suggesting that the proliferative effect of ELAF was indeed mediated by its capacity to activate PKC. Western blot and immunocytochemistry showed the signalling pathways involved in this proliferative effect of ELAF on NPCs.

P16-75
Additive inhibitory modulation of cAMP accumulation in the hippocampus by the adenosine A1 and cannabinoid CB1 receptors is independent of A1 receptor desensitization

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The adenosine A1 and cannabinoid CB1 receptors are highly expressed in hippocampus and inhibit neurotransmitter release through similar transduction pathways. We set forth to determine the type of interaction found between A1 and CB1 receptors activity on cAMP accumulation in the rat hippocampus. The CB1 agonist WIN55212-2 (0.3–30 μM) decreased forskolin-stimulated cAMP accumulation in the hippocampal slice, with an EC_{50} of 6.6 ± 2.7 μM and an E_{max} of 31 ± 2%, whereas for the A1 agonist, N^6-Cyclopentyladenosine (CPA, 10–150 nM), an EC_{50} of 35 ± 19 nM and an E_{max} of 29 ± 5% were obtained. The inhibitory effect of CPA (100 nM) on cAMP accumulation (15 ± 0.6%, n = 3) was not affected by adenosine deaminase (2 U/ml; 21 ± 2.9%, n = 9) and was blocked by the A1 receptor selective antagonist DPCPX, whereas the effect of WIN55212-2 was reversed by the CB1 receptor selective antagonist AM251. Forskolin-stimulated accumulation of cAMP was not affected by DPCPX or AM251. The combined inhibitory effect of nearly maximal concentrations of WIN55212-2 (30 μM) and CPA (100 nM) on cAMP accumulation was 41 ± 6% (n = 4), which did not differ ($p > 0.4$) from the sum of the individual inhibitory effects of each agonist (43 ± 8%, n = 4), but was different ($p < 0.05$) from the effects of CPA (100 nM) or WIN55212-2 (30 μM) alone. Pre-incubation with CPA (100 nM) for 95 min was enough to desensitize the A1-mediated action. The inhibitory effect of WIN55212-2 (30 μM) on cAMP accumulation was not affected by A_1 receptor desensitization. It is concluded that the combined effect of CB1 and A1 agonists on cAMP accumulation is additive and is not affected by A1 receptor desensitization, suggesting that the cAMP signalling pathways triggered by WIN55212-2 and CPA act independently in the hippocampus.

P16-76
Validation of inflammatory activity of cystatin B deficient microglia

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Loss-of-function mutations in the *cystatin B* gene encoding an inhibitor of cysteine proteases cause progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1). Pathogenesis includes early microglial activation that precedes astrocytosis and neurodegeneration. We have investigated inflammatory status of *Cstb*$^{-/-}$ microglia based on expression level of specific inflammatory signalling proteins and efficacy of anti-inflammatory antibiotic minocycline to modulate their activation in *Cstb*$^{-/-}$ mouse *in vitro* and *in vivo*.

Primary microglial cells were prepared from postnatal-day 5 mice and activated by lipopolysaccharide (LPS, 0.5 μg/μl) with/without pretreatment by minocycline (10–25 μM). Morphology, cytokine secretion, relative expression of phospho-p38MAPK, CD68, MHC II, caspases 1 and 3, IL1b, COX2, NOS2, cathepsins B, S were estimated in *Cstb*$^{-/-}$ and control mixed glial cultures. In *in vivo* experiments control and *Cstb*$^{-/-}$ mouse pups were injected with minocycline-HCL (40 mg/kg/day, s.c.). Mouse brains (P14) were analysed for microglial activation. M1 and M2 phenotyping of *Cstb*$^{-/-}$ and control microglia was initiated.

Cstb$^{-/-}$ microglia morphologically are more activated and highly express caspases 1, 3, pp38MAPK, iNOS, cathepsins and secreted IL1b, IL6 and chemokines (CCL5, CCL3, CCL4, CCL2, IP10). Minocycline diminished pp38MAPK activation and iNOS expression in *Cstb*$^{-/-}$ cells, but expression of other inflammatory markers was even elevated (e.g. chemokine production). *In vivo* experiment did not show efficacy of minocycline to alleviate microglial activation in *Cstb*$^{-/-}$ mouse. Preliminary phenotyping results showed M1 polarisation of *Cstb*$^{-/-}$ microglia.

Our results indicate a remarkable role of microglia in cystatin B-deficiency related brain pathogenesis and suggest a role for cystatin B in regulation of immune response. Minocycline as a neuroprotective agent does not effectively alleviate proinflamma-

tory actions of *Cstb*$^{-/-}$ microglia and more specific therapeutic molecular targets are needed to prevent brain inflammation caused by CSTB-deficiency.

P16r-77
Functional genetic analysis of small glutamine-rich tetratricopeptide-repeat protein-alpha (SGT- alpha) in mice

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Nerve terminals have developed a chaperone system to maintain functional protein conformations (e.g. the SNARE protein SNAP25) during the synaptic vesicle cycle. The chaperone complex is composed by cysteine string protein-alpha (CSP-alpha), heat shock cognate protein of 70 kDa (Hsc70) and SGT- alpha (small glutamine-rich tetratricopeptide repeat-containing protein). The function *in vivo* of CSP-alpha have been investigated in Drosophila and mouse. Indeed, mice lacking CSP-alpha develop a neurological phenotype and early death due to activity-dependent degeneration of synaptic terminals. In addition, it has been recently described that mutations in the human gene (DNAJC5) that codes CSP-alpha causes adult-onset autosomal dominant neuronal ceroid lipofuscinosis, a devastating neurodegenerative disease. In contrast, the synaptic function *in vivo* of SGT-alpha remains to be analyzed. Now, we have studied a mouse line generated by gene-trapping targeting of the alpha-SGT gene in mouse ES cells (Texas A&M Institute for Genomic Medicine). The gene-trap is based in a retroviral vector that contains a splice acceptor sequence followed by a promoterless selectable marker such as β-Geo, a functional fusion between the β-galactosidase and neomycin resistance genes, with a polyadenylation signal. We have used polyclonal antibodies raised against the full-length human SGT-alpha to show that the expression of SGT-alpha is abolished in the brain of SGT-alpha knock-out mice. Surprisingly, in contrast to the CSP-alpha KO mice, SGT-alpha KO mice are viable, fertile and do not show any apparent increased morbidity or mortality. Perhaps, the brain-specific SGT isoform, SGT-beta, is able to compensate the lack of SGT-alpha. In order to investigate that hypothesis we are focusing our analysis at the presynaptic terminals of GABAergic fast-spiking neurons which suffer from early degeneration at CSP-alpha KO mice.

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P16-78
In vitro effects of seleno-l-methionine on glioblastoma multiforme cell proliferation and death

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Purpose: Selenium is an extraordinary trace element that can incorporate into amino acid and protein structures. Effects of selenium compounds on different types of cells are composition- and dose-dependent. In this study, we investigated the effect of a selenium compound, seleno-L-methionine, in human glioblastoma cell lines in terms of cell death and cell proliferation.
Material-Methods: We used GMS-10 and DBTRG-05MG GBM cell lines. Cells were treated with seleno-L-methionine (50-1000 μM) for 24, 48, 72 hours. Cell death was assessed with LDH assay and cell proliferation analysis was performed by using WST-1 assay (Roche).
Results: High doses (500 and 1000 μM) were found to induce cell death (for both lines) at 24 hour using the LDH assay. After 72 hour, for the DBTRG-05MG cell line 50 μM seleno-L-methionine resulted in % 2.08 ± 0.45 increase, 100 μM resulted in % 13.26 ± 0.76, 500 μM resulted in % 35.52 ± 1.37, and 1000 μM resulted in % 50.10 ± 2.29 increase in cell death compared with the control. For the GMS-10 cell line at 50 μM seleno-L-methionine did not induce cell death; 100 μM resulted in % 3.99 ± 0.65, 500 μM resulted in %10.0 ± 0.89 and 1000 μM resulted in % 20.96 ± 1.90 increase in cell death compared with the control. WST-1 assay, showed that seleno-L-methionine stimulated the proliferation of cell lines in a dose-dependent manner. For the GMS-10 cell line, low doses (50 and 100 μM) were found to induce proliferation at 24 hour: 50 μM seleno-L-methionine resulted in % 8.145 ± 1.9 increase and 100 μM resulted in % 2.6 ± 1.4 increase in cell proliferation compared with the control. We did not find significant difference between the seleno-L-methionine – treated two cell lines for 24 hour, but for 48 and 72 hour we found significant difference (p-value of < 0.05). Student's the test was used for statistical analysis and $p < 0.05$ was considered to represent significant differences between the two group's proliferation status.
Conclusions: Seleno-L-methionine induced cell death on high doses but increased cell proliferation on low doses. This study will yield further studies focused on the possibility of using selenium against some types of GBM.
Keywords: Seleno-L-methionine, glioblastoma multiforme, cell proliferation, cell death

P16-79
Synaptic dysfunctions at the dentate gyrus in CSP-α knock-out mice, a mouse model of neuronal ceroid lipofuscinosis

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Cysteine String Protein-α (CSP-α) is a synaptic vesicle protein required for the long term maintenance of nerve terminals. Interestingly, it has been recently described that mutations in the human gene (DNAJC5), that codes CSP-α, causes adult-onset autosomal dominant neuronal ceroid lipofuscinosis (NCL4) (Noskova et al., 2011). The clinical onset of NCL4 is at about 30 years of age, with patients developing progressive dementia, ataxia, seizures, and myoclonus. That suggests that GABAergic synaptic transmission might be compromised in those patients. On the other hand, hippocampal parvalbumin-expressing GABAergic interneurons, largely comprised of basket cell, are fast spiking interneurons that release neurotransmitter with high speed. They form synaptotagmin2-expressing synapses onto granule cells at the dentate gyrus. Those synapses are particularly sensitive to the absence of CSP-α consistent with the notion that CSP-α has an important role in preventing activity dependent

neurogeneration of synapses (García-Junco-Clemente et al., 2010; Sharma et al., 2011). However, the functional properties of GABA release at those synapses have not been yet characterized in CSP-α KO mice. Here we have used acute hippocampal slices from postnatal age P19-P24 to find out that the properties of GABArelease from basket cells are significantly different in CSP-α KO mice in comparison to littermate controls. In addition we have found changes in the expression of synaptic and cell division markers that suggest compensatory modifications within the dentate gyrus neuronal circuit. We expect that our study will be helpful to get further insights into the functional changes that interneurons and related circuits undergo at the initial stages of synaptic degeneration.

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P16-80
Early changes associated with aging in substantia nigra pars compacta

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Substantia nigra pars compacta (SNpc) is highly sensitive to normal aging and is selectively degenerated in Parkinson's disease (PD). Until now, molecular mechanisms behind SNpc aging have not been fully investigated using high throughput techniques. Here, we show early signs of aging in SNpc, which are much more obvious than in ventral tegmental area (VTA), a region adjacent to SNpc but less affected in PD. Aging-associated early changes in transcriptome were investigated comparing late middle-aged (18 months old) to young (2 months old) mice in both SNpc and VTA. A meta-analysis of published microarray studies resulted in the generation of a common 'transcriptional signature' of the aged (24 months old) mouse brain. SNpc of late-middle aged mice shared characteristics with the transcriptional signature, suggesting an accelerated aging in SNpc. Age-dependent changes in gene expression specific to SNpc were also observed, which were related to neurological functions and inflammation. As a proof of principle, the role of two age-regulated genes in the normal function of SNpc cells are being studied in detail. This study will aid to understand the mechanism of SNpc aging and its potential contribution to age-related disorders like PD.

P16-81
Bioenergetic impairment in Italian Parkinson's disease patients with PARK2 mutations

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There is substantial evidence pointing to a role of mitochondrial dysfunction in the pathogenesis of sporadic and early onset PD. The PARK2 gene encodes Parkin, a protein associated with the outer mitochondrial membrane that functions as an E3 ubiquitin ligase. Parkin participates in the quality control of mitochondrial proteins, autophagy and mitochondrial structural dynamics. Pathogenic Parkin mutations are distributed throughout the Parkin gene and include missense, nonsense and frameshift mutations, as well as exon deletions and multiplications. We investigated on the impact of different Parkin mutations on mitochondrial function, analyzing human primary dermal fibroblasts from Italian patients affected by early onset Parkinson's disease carrying the following mutations: ex3-4del; Cys253Tyr/ex5del; ex2del/ex2-4del. We found a significant decrease in patients' fibroblasts of complex I, IV and V specific activity and a significant reduction of the uncoupled respiration rates independent of the substrate used as compared to controls. Oligomycin-sensitive ATP hydrolase and ATP synthase activity of CV measured in the mitoplast fraction revealed a decrease in the patients' cells as compared with controls. Cellular total ATP content, measured under basal conditions or under strict glycolytic conditions, indicates that the defective ATP production by OXPHOS is compensated by glycolytic supply. Mitochondrial dysfunction in PD patients is associated with a significant decrease in ψ and increase in ROS production. The pattern and severity of mitochondrial dysfunction varying between different mutations, correlated with the clinical features, providing evidence that this dysfunction in Parkin mutant fibroblasts, is involved in the pathogenesis of Parkinson.

P16-82
Role of the RNA-binding protein HuR in neurofibromas and malignant peripheral nerve sheath tumour

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas that arise within the peripheral nerve with very poor prognosis. MPNSTs are sporadic or arise in individuals with neurofibromatosis type 1 (NF1). NF1 patients develop benign dermal neurofibromas and/or benign plexiform neurofibromas, which can undergo malignant transformation to MPNST. Schwann cells are the crucial pathogenic cell type in NFs. Genome-wide gene expression and DNA methylation profiling have shown significant differences between normal Schwann cells, NF1-derived benign and malignant cells.

During tumour development, cancer cells acquire novel physiologic changes that allow them to develop, adapt, survive, proliferate and metastasize by expressing a distinct catalogue of proteins. In addition to genetic and epigenetic mechanisms, post-transcriptional regulation of gene expression by RNA-binding proteins (RBP) also strongly influences the aberrant expression of many of these proteins. The RBP HuR is aberrantly expressed in several types of cancer and a strong correlation has been found between its expression levels and advancing stages of malignancy.

Recently, we examined the role of HuR during Schwann cell development and found that HuR was highly expressed in immature Schwann cells. Using RNA immunoprecipitation (RIP) coupled with microarray analysis (RIP-ChIP), hundreds of its target mRNAs were identified. Gene Ontology (GO) classification showed that HuR bound to several functionally related mRNAs, regulating proliferation, apoptosis and morphogenesis (Iruarrizaga *et al.*, 2012). Here, GO classification of these target mRNAs showed that cancer was one of the top enriched categories with 49 significantly enriched genes. Amongst the genes significantly regulated, several one with well-defined roles in oncogenesis were identified, including *Cyclin D1, Cyclin D2, NRAS, Cyclin A, Sox9 and Ncam1*. We found that HuR expression was significantly increased in NF (n = 105) and MPNST (n = 34) samples

compared to normal nerves, with a strong correlation between HuR expression and degree of malignancy. Using MPNST cell lines, we found that HuR was bound to several of these cancer-associated mRNAs. HuR silencing significantly reduced the expression of these genes, made these cells more sensitive to apoptotic death by UV irradiation, and proliferation, migration and invasion were also reduced in these cells. These results suggest that control of gene expression by HuR could be an important determinant in regulating the oncogenic charateristics of cancer Schwann cells.

P16-83
Geminin regulates neural progenitor cells self-renewal and differentiation decisions in the mouse cortex

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Neural stem cells (NSCs) can be identified both in the embryonic and adult brain, and generate neurons, astrocytes and oligodendrocytes. In early embryogenesis, neuroepithelial cells (NEs), are localised in the ventricular zone (VZ) of cortex, where they undergo proliferative divisions and expand their numbers. Progressively, NEs give rise to radial glial cells (RGCs) that reside in the VZ, and generate the basal progenitors (BPs), which colonize the subventricular zone (SVZ).

In the adult brain, NSCs reside in the subgranular zone (SGZ) of the hippocampus and the subependymal zone (SEZ) of the lateral ventricles. Quiescent NSCs in SEZ give rise to transit-amplifying progenitors, which generate oligodendrocytes that migrate to corpus callosum, and neuroblasts that migrate rostrally and differentiate into olfactory bulb interneurons. On the contrary, NSCs of the adult dentate gyrus (DG) are generated in the SGZ of the DG and differentiate into neuronal and glial cells in the granular layer of the DG.

Our previews work has shown that Geminin overexpression reduces neural progenitors by forcing them to exit the cell cycle towards premature differentiation. We generated mice lacking Geminin expression in the CNS. Our data show that early cortical progenitors lacking Geminin exhibit a longer S-phase which leads to a transient expansion of neural progenitor cells in the developing cortex. Additionally, Geminin deletion alters the output of deep and upper layer neurons. Furthermore, adult NSCs in the absence of Geminin alters the number of adult quiescent NSCs in the SEZ.

Our results suggest that Geminin regulates proliferation and differentiation decisions in cortical progenitor cells by influencing cell cycle kinetics and the type of division of neural progenitor cells.

P17 – Nitrogen Biochemistry and Metabolism

P17-1
The use of 'omics' approaches to assess the role of plastidic glutamine synthetase in plant metabolism

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The study of mutants lacking the plastidic isoform of glutamine synthetase (GS2) has demonstrated the involvement of this enzyme in the reassimilation of photorespiratory ammonium [1] [2], nodulation [3] and other processes [4] in the model legume *Lotus japonicus*. Different 'omics' approaches have been now used with these mutants in order to obtain a more general view of the role of GS2 in *L. japonicus* metabolism. A combination of transcriptomic, metabolomic and qRT-PCR data will be presented to show that: (i) the lack of GS2 has a deep impact on the central metabolism under photorespiratory active conditions, (ii) *L. japonicus* photorespiratory and photosynthetic genes are regulated in a coordinate way, (iii) GS2 plays a fundamental role in the response to drought stress and is involved in proline production, (iv) GS2 is involved in the transcriptional response of the plant to the presence of different nitrogen sources and (v) GS2 shares a similar transcriptional profile with several other genes of carbon metabolism.

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P17-2
Specific molybdate transporters and molybdoenzymes in Chlamydomonas

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Molybdenum is an oligoelement that in form of a molybdopterin cofactor (Moco) participates in the active site of key molybdoenzymes such as nitrate reductase, sulfite oxidase, xanthine dehydrogenase and amidoxime reductase. By insertional mutagenesis, mutants affected in each of the steps for Moco biosynthesis were isolated but not in the molybdate transport step. Two high affinity molybdate transporters, MOT1 and MOT2, were identified in Chlamydomonas from transporters of unknown functions. They define two new families of transporters. Their functionality was demonstrated by an antisense strategy and by their heterologous expression in Saccharomyces. MOT1 expression depends on *NIT2* the major gene for nitrate assimilation, whereas that of MOT2 is stimulated by nitrate in Mo-deficient medium.

The last molybdoenzyme discovered prevents toxicity of amidoximes by a redox system analogous to the one present in humans consisting of cyt b5 reductase, cyt b5-1, and the molybdoenzyme reducing component, that binds MoCo by the conserved cysteine 252.

P17-3
The glutamine synthetase gene family in Populus

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Background: Glutamine synthetase (GS; EC: 6.3.1.2, L-glutamate: ammonia ligase ADP -forming) is a key enzyme in ammonium assimilation and metabolism of higher plants. The current work was undertaken to develop a more comprehensive understanding of molecular and biochemical features of GS gene family in poplar, and to characterize the developmental regulation of GS expression in various tissues and at various times during the poplar perennial growth.

Results: The GS gene family consists of eight different genes exhibiting all structural and regulatory elements consistent with their roles as functional genes. Our results indicate that the family members are organized in four groups of duplicated genes, three of which code for cytosolic GS isoforms (GS1) and one which codes for the choroplastic GS isoform (GS2). Our analysis shows that *Populus trichocarpa* is the first plant species in which it was observed the complete GS family is duplicated. Detailed expression analyses have revealed specific spatial and seasonal patterns of GS expression in poplar. These data provide insights into the metabolic function of GS isoforms in poplar and pave the way for future functional studies. By other hand we produce the corresponding recombinant proteins for each isogene in order to study molecular characteristic and kinetic parameters. This work could help to understand the dependence of the physiological behaviour of each enzyme from the molecular properties.

Conclusions: Our data suggest that GS duplicates could have been retained in order to increase the amount of enzyme in a particular cell type. This possibility could contribute to the homeostasis of nitrogen metabolism in functions associated to changes in glutamine derived metabolic products. The presence of duplicated GS genes in poplar could also contribute to diversification of the enzymatic properties for a particular GS isoform through the assembly of GS polypeptides into homo oligomeric and/or hetero oligomeric holoenzymes in specific cell types.

P17r-4
Induction of alternative pathways for the reassimilation of photorespiratory ammonium in glutamine synthetase mutants from *Lotus japonicus*

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The photorespiratory cycle is an important metabolic route which takes place in plants between chloroplasts, mitochondria and peroxisomes. The study of mutants lacking different enzymes

of this route is fundamental in order to determine the exact physiological function of photorespiration, a topic that is still object of debate [1]. Mutants from the model legume *Lotus japonicus* that lack of plastidic glutamine synthetase (GS2) [2] were used recently to determine the key role of plastidic glutamine synthetase (GS2) in the reassimilation of the ammonium generated by the photorespiratory cycle. Under photorespiratory active conditions, these mutants accumulate high levels of ammonium, reaching a maximum at three days followed by a sudden decrease of NH_4^+ levels. We have further investigated if this decrease in ammonium levels might be due to the induction of alternative ammonium assimilatory pathways in this plant. For this purpose, the levels of transcript, protein and enzyme activity of different ammonium-utilizing enzymes was determined at different times under active photorespiration. Moreover, the metabolome of wild type and mutant plants was obtained under the same conditions. The results obtained indicated that different isoforms of cytosolic glutamine synthetase, glutamate dehydrogenase and asparagine synthetase were induced under active photorespiration in the mutant plants, and may be involved in the reassimilation of photorespiratory ammonium. The study of the metabolome permitted also to analyze the fate of ammonium re-assimilated by these enzymes.

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P17-5
Insights into membrane association of *Klebsiella pneumoniae* NifL under nitrogen-fixing conditions from mutational analysis

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In the free-living diazotroph *Klebsiella pneumoniae* nitrogen fixation is regulated by NifA and its antagonist NifL in response to molecular oxygen and nitrogen availability. Under anaerobic conditions and nitrogen limitation the N-terminal located FAD cofactor of NifL is reduced by electrons from the menaquinol pool of the anaerobic respiratory chain leading to the sequestration of NifL to the membrane and allowing cytoplasmic NifA to activate transcription of the nitrogen fixation (*nif*) genes. Here we present a genetic screen aiming to identify amino acids of NifL essential for sequestration to the cytoplasmic membrane under nitrogen fixing conditions. Based on NifL mutants obtained in the screen several point mutations were introduced into *nifL* by site directed mutagenesis, and the respective effects on NifA-mediated *nif*-gene induction were monitored in *K. pneumoniae* using a *nifK'-'lacZ*-fusion. Selected NifL derivatives which resulted in significant *nif*-gene inhibition were further analysed concerning membrane association and FAD binding capability using biochemical approaches.

P17r-6
Regulation of the recycling of the Ammonium derived from lignin synthesis involves Myb factors

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The recycling of nitrogen in the xylem of trees it's a quantitative important process due to the big amount of ammonium that is liberated in the deamination phenylalanine by the phenylalanine ammonia lyase reaction, the first enzyme of the phenylpropanoids pathway (Humphreys and Chappla, 2002). This ammonium is reincorporated by GS1b a Glutamine Synthetase isoform localized in the xylem of pine trees (Avila et al 2001). On the other hand, Aspartate aminotransferase catalyzes a reaction where ammonium is incorporated into the route of phenylpropanoids via arogenate (Maeda H, 2010). To make this process efficient this enzymes must be co-regulated. We have seen that these three genes have a similar pattern of expression, with higher levels in vascular tissues. Considering that the whole process could be regulated by Myb factors, we thought that PtMyb1, PtMyb4 and PpMyb8 could be good candidates for the co-regulation of the process for some reasons: (i) its expression pattern is similar, (ii) they bind to AC elements contained in the AAT, GS1b and PAL promoters and (iii) these transcription factors act as activators of the expression of these genes in transient expression experiments using shoot pine protoplasts. Related experiments to proof this hypothesis will be presented.

P17r-7
Desulfovibrio gigas NorR-like under nitrosative stress

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Transcriptional regulation in anaerobic bacteria under nitrosative stress appears to involve different nitrogen oxide transcription factors, including NorR. Work performed in our laboratory has shown that in *Desulfovibrio gigas* flavodiiron protein rubredoxin:oxygen oxidoreductase (ROO) provides protection against nitrosative stress. It is described that the transcriptional regulation of *roo* orthologs in other organisms involves the transcription factor NorR. We have identified in the ROO promoter region upstream of the ATG, two sites with the consensus GT-(N_7)-AC which most probably can bind NorR. It is thus plausible to put forward the hypothesis that NorR of *D. gigas* is involved in the regulation of *roo*. In order to identify the function of NorR we first search the *D. gigas* genome with orthologs of other microoganisms. We found two potential sequences that after analysis only one contained the sequence LKKARG corresponding to the DNA binding domain. The typical modular structure of the members of NtrC/NifA family in which NorR is comprised was also found in the one of *D. gigas*. The gene encoding NorR was deleted of its genome by homologous recombination using the kanamycin reporter. The growth of this mutant and of *roo* strain is severely impaired in the presence of the NO generator, the GSNO 10 μM. This result suggests that NorR is possible involved in NO detoxification of *D. gigas* and is probably responsible for the regulation of *roo* expression. We also show that *E. coli* Δ*norR* grows slower than the wild-type strain in the presence of GSNO 500 μM. We have complemented the *E. coli* Δ*norR* with the *norR* gene of *D. gigas* and compared its growth with *E. coli* wild-type strain 6 hour after NO treatment. Both strains have similar growth rates indicating that most probably

NorR is playing a role similar to its counterpart in *E. coli*. We are now purifying the NorR protein to perform electrophoretic mobility binding assays to examine if NorR recognizes the consensus sequence found in the promoter of *roo*.

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P17-8
Assimilation of cyanide and synthesis of bioplastics by *Pseudomonas pseudoalcaligenes* CECT5344

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Cyanide is one of the most potent and toxic chemicals produced by industry. The jewellery industry generates a waste-water (residue) that contains free cyanide as well as large amounts of cyano-metal complexes. The bacterium *Pseudomonas pseudoalcaligenes* CECT5344 tolerates and assimilates high cyanide concentrations (up to 30 mM) under alkaline conditions (pH 9–10), thus avoiding its volatilization as HCN. This strain is a model organism to be used in bioremediation processes and biotreatment of industrial residues containing cyanide and its derivatives. In this work we demonstrate that *P. pseudoalcaligenes* CECT5344 is able to accumulate granules of polyhydroxyalkanoates (PHAs) in its cytoplasm with sodium octanoate and cyanide-containing residue as carbon and nitrogen sources, respectively. PHAs have attracted research interest because they are biobased and biodegradable thermoplastics that have the potential to replace petrochemical plastics. The PHA granules accumulated by this bacterium were observed by transmission electron microscopy. Genes involved in the biosynthesis and metabolism of scl-PHAs (monomers consisting of 4–5 carbon atoms) and mcl-PHAs (monomers from 6 to 14 carbon atoms) were found to be located in three different loci in the genome of *P. pseudoalcaligenes* CECT5344. Three mutant strains of *P. pseudoalcaligenes* affected in PHA biosynthesis were generated and cultured in a batch reactor. These mutants are Δ*phaC1ZC2* that only accumulates scl-PHA, Δ*phaC* that only synthesizes mcl-PHA and the double mutant Δ*phaC1ZC2*/Δ*phaC* that was unable to accumulate PHA. The storage material accumulated by wild type and mutant strains were extracted and characterized by gas chromatography–mass spectrometry (GC-MS).

P17r-9
DNA binding, allosteric regulation and PipX coactivation clarified structurally for the NtcA global nitrogen regulator of cyanobacteria, which belongs to the Crp-Fnr superfamily of transcription factors

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Transcription regulators of the Crp-Fnr superfamily are key players for bacterial gene expression regulation. Among them, NtcA orchestrates in cyanobacteria the gene expression response to nitrogen starvation, commanding a large regulon, being activated by two oxoglutarate (2OG, a nitrogen starvation signaling molecule) and coactivated by protein PipX. We illustrate the constancy of the regulator-DNA complex structure within the Crp-Fnr superfamily by determining crystal structures of NtcA, bound to its target DNA sequence. In addition, we provide with NtcA a unique glimpse of the architecture of a Crp-Fnr-family factor being bound simultaneously to target DNA, small molecule activator (2OG) and protein modulator (PipX), by determining the corresponding crystal structure for this quaternary complex. We also prove that the structures of 2OG-activated NtcA alone or bound to DNA are identical except for changes in the conformations of side chains of DNA-interacting residues. PipX links NtcA regulation with nitrogen control mediated by the signaling protein PII. Under conditions of nitrogen abundance, PII sequesters PipX, rendering this coactivator unavailable for NtcA activation. Our structure of NtcA bound to both 2OG and PipX led us to conclude that PipX coactivates NtcA by stabilizing the active conformation of NtcA. However, it was very important to ascertain whether PipX mediates in part its coactivation role by interacting with DNA. The present structures of the DNA-NtcA and PipX-NtcA-DNA complexes exclude such interaction of PipX with the DNA, which is highly bent (approximately 80°) by the transcription factor. These structures fully clarify the mechanism for the exquisite specificity of NtcA for its target DNA sequence, for which we provide mutational evidence. Such specificity is essential for discrimination between the NtcA box and the highly similar Crp box, since Crp and NtcA coexist in cyanobacteria, and each one of them controls a different regulon. Projects BFU2011-30407 of MEC Spain, and Prometeo of Generalitat Valenciana.

P17-10
Biochemistry and biotechnology of cyanide assimilation in *Pseudomonas pseudoalcaligenes* CECT5344

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Effluents from industries such as mining and jewelry contain free cyanide, metals and cyano-metals complexes that constitute an important environmental problem. Cyanide may be a suitable nitrogen source for those microorganisms able to produce a cyanide-resistant respiratory chain and to grow under iron-limited conditions since cyanide strongly binds iron and other metals. The alkaliphilic bacterium *Pseudomonas pseudoalcaligenes* CECT5344 has the capacity to growth in media with free cyanide by inducing a cyanide-insensitive alternative oxidase coupled to a malate:quinone oxidoreductase activity that oxidizes L-malate to oxaloacetate. Oxaloacetate reacts chemically with cyanide to produce a cyanohydrin (2-hydroxynitrile), which can be further converted into ammonium by the nitrilase NitC. By a proteomic approach several cyanide-induced proteins encoded by *nitB*, *nitG*, *orf1* and *serC* have been identified. The genome sequence of the strain CECT5344 has allowed to study the genetic context of these genes, indicating the presence of gene clusters involved in cyanide assimilation (*nit1C*) and resistance (*cio1*). The analysis of the draft genome sequence of *P. pseudoalcaligenes* CECT5344 has revealed the presence of genes probably involved in siderophores metabolism as well as genes related to other processes with a great biotechnological potential like production of bioplastics, biodegradation of pollutants or resistance to heavy metals. Proteomic analysis also revealed that some cyanide-induced proteins are involved in cyanate metabolism and oxidative stress

response, as well as transporters and ribosomal proteins, suggesting that cyanide causes a great variety of changes in the metabolism of the strain CECT5344. All these findings led us to have a wide knowledge about the cyanide metabolism in *P. pseudoalcaligenes* CECT5344 to be applied in the cyanide bioremediation of a cyanide-containing industrial waste in a chemiostat.

P17-11
Role of halophilic archaea in N-cycle at the salty environments

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The past decade has seen a rekindling of interest in extreme microorganisms isolated from ancient evaporates and salty environments such as multipond salterns (which are usually semi-artificial coastal or inland systems), marshes or salted lakes. From a practical point of view, there is concern that viable microbial communities in salt deposits could lead to biodeterioration of containers holding toxic and nuclear waste. On the other hand, there is high interest on biotechnological uses of haloarchaea and their molecules in biotechnology. However, the knowledge about their physiology, metabolism or the role they are playing within the biogeochemical cycles is scarce.

Natural salty ecosystems are nice and very interesting model systems to investigate the influence of salinity on microbial diversity and productivity. The microorganisms living in these environments, called halophiles, are found in each of the three domains: Archaea, Bacteria and Eukarya. However, red halophilic Archaea from the family Halobacteriaceae dominate in these environments. New highlights on N-cycle have revealed that these halophilic microorganisms sustain the N-cycle in the cited environments, especially thanks to the assimilatory nitrate reduction, denitrification and ammonia oxidation. In that context, denitrification is revealed as the most important pathway because denitrifying haloarchaea are able to uses several final electron acceptors (nitrate, nitrite, fumarate, perchlorate, chlorate or dimethyl sulfoxide). Besides, some of the denitrifying haloarchaea show high range of tolerance to nitrogenous compounds such us nitrite, which is extremely toxic for most of the living systems. This ability allows the use of these haloarchaea as excellent models to optimize waste water treatments.

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P17-12
Effect of nitrate concentration on relative abundance of *narG, napA, nirK, nirS* and *nosZ* bacterial denitrifying genes in sediments from the Rocina Stream (Doñana National Park)

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Nitrate contamination of waters and sediments is continuously increasing due to antropogenic activities. After leaching from soils, nitrate reaches rivers and lakes and, finally, seas and oceans. However, our knowledge on the effect of nitrate concentration on the denitrifying bacterial community in sediments is scarce. After screening more than 20 sites along the Rocina Stream (Doñana National Park) two sites were selected with low and high nitrate concentration, Laguna del Acebrón (S1) and Arroyo de la Cañada (S2), respectively. Samples of sediments were taken in April and October 2008–2010.

DNA was extracted from the sediments and the size of the denitrifying community was estimated by quantification of the *narG*, *napA*, *nirK*, *nirS* and *nosZ* denitrifying genes by real-time PCR (qPCR). Size of the total community was estimated using the 16S rRNA gene as a molecular marker. Denitrifying enzyme activity (DEA) of the sediments was measured by N_2O production.

Regardless of the sampling year and month, nitrate concentration was significantly higher in S2 than in S1 according to the Mann-Whitney test. Except for *nirK*, relative abundances of the remaining denitrification genes were always higher in S2. Whereas no differences in DEA were observed in sediments taken at S1 in April and October, values in sediments from April were always higher than those taken in October at S2. Except for the *nirK* gene, a high correlation between each one of the denitrifying genes and the nitrate concentration was found when using the Spearman test. The highest correlation was found for the *nosZ* gene. No correlation was observed between DEA and any of the denitrifying genes.

A principal component analysis of the variables analyzed in this study projected the individuals from S1 and S2 in two different spaces, which indicates they have different tendencies.

A linear regression model using pH, nitrate concentration and gene abundances as independent variables allowed us to predict values of DEA with an R^2 of 0.823.

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P17-13
Recombinant glutamine synthetases from Haloferax mediterranei. Studies of characterization and possible interactions between them

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Haloferax mediterranei is an extreme halophilic microorganism belonging to the Archaea Domain isolated from Santa Pola solar salterns (Alicante, Spain) in 1983. This heterotroph is able to grow with nitrate or ammonium as nitrogen sources. To use NO_3^- it must be reduced to NH_4^+, which is then incorporated to carbon skeletons via Glutamate Dehydrogenase under nitrogen-sufficient conditions; or Glutamine Synthetase-Glutamate Synthase (GS-GOGAT) under nitrate or nitrogen-starvation conditions. The GS catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonium in the presence of divalent metal ions (biosynthetic GS activity). It also catalyzes the production of γ-glutamyl hydroxamate and free ammonium from hydroxylamine and glutamine in the presence of a nucleotide, arsenate and a metal ion (transferase GS activity). In the draft genome of *H. mediterranei* (M.J. Bonete et al., unpublished), three genes have been identified (*gln*A, *gln*A-2 and *gln*A-3) with homology to glutamine synthetase. Based on the presence of three consensus sequences used to identify GS, it is observed that one of the genes contains the three conserved domains (*gln*A). So, it will be studied the possible interaction between the three different proteins using Isothermal Titration Calorimetry (ITC). The three genes of halophilic GS were heterologously overexpressed in *E. coli* (pET3a). These proteins were obtained as inclusion bodies and they have been purified in one step using DEAE-cellulose chromatography. For biosynthetic activity from

recombinant GS, K_ms for glutamate, ATP and ammonium have been calculated. Also it has been studied the effect produced by 2-oxoglutarate, Mn^{2+} and Mg^{2+} in glutamine synthetase activity. For recombinant GS transferase activity, K_ms for hydroxylamine, glutamine and ADP have been calculated. Finally, the effect of protein dilution on its enzymatic activity was studied. This work was supported by research project BIO2008-00082 from MEC (Spain).

P17-14
Differential expression of asparagine metabolism genes in *Lotus japonicus* plants

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Asparagine is an amino acid amide that has a N:C ratio of 2:4, which makes it an efficient molecule for the storage and transport of nitrogen within plants [1]. This is the case in the model legume *Lotus japonicus* where asparagine is the principal molecule used to transport reduced nitrogen and can account for 86% of the nitrogen transported from root to shoot, under sufficient nitrogen supply conditions [2]. In this plant, structural analysis of K + dependent and K + -independent asparaginases was recently carried out [3]. We report here the gene expression analysis of different genes involved in asparagine metabolism. The results obtained indicated different patterns of expression of asparagine related genes in different plant organs of *L. japonicus* plants. High levels of expression of asparagine synthetase were detected in mature leaves, in contrast with the results obtained in young leaves, thus indicating the key role of mature leaves as a source tissue of asparagine in this plant, together with roots and nodules which also showed high levels of asparagine synthetase expression. In addition, the comparative analysis of serine-glyoxylate amino transferase expression in wild type and asparaginase and glutamine synthetase photorespiratory mutants established an interconnection between asparagine metabolism and the photorespiration process in this plant.

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P17r-15
Insight on nitrogen signaling in a high-salt world: crystal structures of PII protein from the halophilic archaeon *Haloferax mediterranei*

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In all life domains homotrimeric PII proteins play key signaling roles in the adaptation to nitrogen abundance changes, interacting with protein targets mainly via their flexible T-loops. Of 28 organisms for which PII structures were determined, only two are archaea. We now report crystal structures at 2.6Å and 1.8Å resolution of PII for a third archaea, the extreme halophile *Haloferax mediterranei* (*Hm*PII), opening the way for analysis of high salt adaptation in PII signaling. In line with known preferences of halophilic organisms, a number of exposed PII residues that are well conserved in most species are replaced by glutamate or aspartate in *Hm*PII. Another exclusive trait of *Hm*PII is an 11-residue mainly acidic N-terminal extension that is found only in the PII sequences from halophilic organisms and that in the *Hm*PII structure runs parallel to the threefold axis, protruding over the PII body and extending its surface by 10%, being involved in inter-PII contacts leading to hexamers in one of our crystals. This crystal had ATP (ATP and ADP are classical PII ligands) bound to PII at the T-loop base. In the other crystal ADP binds at this site although no nucleotide was added. 2-Oxoglutarate (2OG), an ammonia poorness signal, is a key allosteric effector of PII, binding to it cooperatively with ATP. In our PII-ADP complex an electron density mass sits at the 2OG site, suggesting that in *Hm*PII 2OG might bind together with ADP. Although in most PII structures the T-loop flexibility prevents T-loop tracing, one T-loop in our ATP-containing crystal is well defined and adopts a novel 2.5-turn alpha-helical fold, raising the question of whether the T-loop might interact with its targets in this alpha helical conformation. Projects BFU2011-30407 and BIO2008_00082 of the Spanish Ministry of Science and Prometeo of the Valencian Government.

P17-16
Arrestins mediate in Ynt1 endocytosis

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Arrestins are involved in ubiquitination and subsequent internalization of several transporters in yeast. We are interested in studying the role of the protein family ART (arrestin-related trafficking adaptors) in ubiquitination and internalization of the high affinity nitrate transporter Ynt1 in the yeast *Hansenula polymorpha*. The disruption of the five genes encoding for arrestins reveals the implication of all of them in Ynt1 degradation. In Ynt1 ubiquitination in response to glutamine, HpArt3 seems to have a predominant role. However, in response to the absence of carbon all the arrestins appear to be involved. These results suggest that all the arrestins participate to a greater or lesser extent in the process of Ynt1 ubiquitination. Overexpression of each of the arrestins in a strain lacking the five would show the absence of specificity for the cargo and rather point to a stimulus specificity.

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P17-17
Mutational analysis provide insights into the functions of the cyanobacterial regulator *PipX*

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The cyanobacterial regulator PipX provides a functional link between the global transcriptional regulator NtcA and the signal transduction protein PII (encoded by *glnB*), a protein found in all three domains of life as integrators of signals of the nitrogen and carbon balance. PipX can form alternative complexes with NtcA and PII and these interactions are respectively stimulated and inhibited by 2-oxoglutarate, providing a mechanism by

which PII can modulate expression of the NtcA regulon, activated at relatively high levels of intracellular 2-oxoglutarate. Structural information on PipX-NtcA complexes suggests that PipX coactivates NtcA controlled genes by stabilizing the active conformation of NtcA bound to 2-oxoglutarate, and by possibly helping to recruit RNA *polymerase*.

Although the *glnB* gene is essential in cultures of S. *elongatus*, *glnB* inactivation is possible in the presence of loss of function mutations of *pipX*, a finding supporting the *in vivo* importance of PII-PipX complexes. To gain further insights into *PipX* functions, we performed a mutational analysis of *pipX* informed by the structural data. Two amino acid substitutions (Y32A and E4A) were of particular interest, since they activated NtcA dependent genes *in vivo* at lower 2-oxoglutarate levels than wild type PipX. While both mutations impaired complex formation with PII, only Y32A had a negative impact on PipX-NtcA interactions. Recent data on genetic analysis of double mutants (PipXY32A/NtcA-, PipXE4A/NtcA-, PipXY32A/PII- and PipXE4A/PII-) support the possibility of NtcA-independent activation of nitrogen regulated genes mediated by such PipX variants.

P17-18
Functional genomics of nitrogen metabolism in conifer trees

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Forest trees dominate large ecosystems and include a broad variety of plant species, some ranking as the largest, tallest, and longest living organisms on Earth. Conifers are the most important group of gymnosperms, which include tree species of great ecological and economic importance that dominate large ecosystems and play an essential role in global carbon fixation. Functional genomics studies are needed to understanding fundamental conifer biology and adaptation of conifer trees to environmental conditions.

Nitrogen is usually available at extremely low levels in forest ecosystems. Consequently, along evolution conifers have developed adaptation mechanisms to cope with mineral stresses, including mycorrhizal associations to increase the efficiency of nitrogen acquisition and metabolic assimilation. However, industrial activities, atmospheric contamination and intensive agricultural practices have increased nitrogen deposition in the ecosystems leading to nutrient imbalance and decreased growth.

Ammonium is the predominant source of nitrogen in the soil of forests, and it is well documented that conifers, unlike herbaceous plants, have a preference for ammonium over nitrate as an inorganic nitrogen source. Research efforts in our laboratory are addressed to understand the response of conifers to ammonium availability (excess/deficiency) and the regulation of ammonium assimilation into amino acids. Our work focus in maritime pine (*Pinus pinaster* Aiton), a forest tree species of great economic and ecological importance, and the most important conifer model for genomic research in Europe. We are using transcriptomic and other functional analysis to examine the response to nitrogen nutrition and how changes in primary metabolism can influence plant growth and developmental programmes. An overview of our research activities will be presented and discussed. Research supported by Spanish Ministry of Education and Science (Grants BIO2009-07490 and PLE2009-0016).

P17m-19
Problems in the study of the nitrogen metabolism in the metabolic syndrome

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Protein utilization by humans and its role in malnutrition has received considerable attention in the past. However, when compared with lipids or carbohydrates, we know very little about amino acid metabolism, especially in relation to overall energy metabolism. Studies on amino-N metabolism were done to explain the emaciation of fasting and malnutrition; but under conditions of abundant food supply: the metabolic syndrome and related diseases practically nothing has been studied in depth. The problem today is compounded by the high dietary protein consumption worldwide, and the progressive use of hyperproteic diets, which effects so far are largely unknown. Under conditions of excess energy (lipids) the priority is lipid oxidation in peripheral tissues, sparing glucose; this mechanism designed for starvation results in insulin resistance. But under food deprivation, amino acid hydrocarbon skeletons are used for energy and, especially, gluconeogenesis, in a way that the presence of sufficient glucose/energy spares amino acids. Then, in the metabolic syndrome the problem is compounded: high energy and protein intake, lipids spare glucose, which high levels spare amino acids. Urea excretion is diminished, and protein deposition is limited. Then, where do the excess amino-N go?, and through which pathways? We are prepared for starvation, not for an excess of nutrients, and we have evolved to save and preserve amino acids. Thus, how are we solving the conundrum of excess energy/ dietary protein? It is time we focus on this practically untouched field and advance our understanding of the mechanisms regulating the homoeostasis of N in health and disease.

P18 – Nutritional Biochemistry

P18-1
Further study of genes involved in mouse steatotic liver identified by DNA microarray

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Using nutritional and genetic models of hepatic steatosis in mice together with microarray analysis and its confirmation by qPCR we have identified a series of genes whose expression is associated with hepatic steatosis as well as potential nutritional strategies able to cope with it. The expression of 10 genes (*Fsp27, Aqp4*,*Cd36, Ly6d, Scd1, Hsd3b5, Syt1, Cyp7b1*, and *Tff3*) showed a significant association in apoEKO mice fed a Western type diet enriched with linoleic acid isomers. T*rans*-10,*cis*-12-CLA enriched diets promote fatty liver in mice while *cis*-9,*trans*-11 reduce it. Those genes were also analyzed in hyperhomocysteinemic mice lacking *Cbs* gene where only *Fsp27,Cd36, Scd1, Syt1* and *Hsd3b5* mRNA expressions were associated with liver steatosis. In apoEKO mice fed an olive oil enriched diet displaying fatty liver reduction, only *Fsp27* and *Syt1* expressions were found associated. Furthermore, Fsp27 mRNA changes were confirmed at protein level although the hepatic protein displayed a molecular mass higher than in adipose tissue suggesting a posttranslational modification. Those results highlight new processes and proteins involved in lipid handling in liver and new strategies to cope with hepatic steatosis.

P18-2
Systemic redox modification after Sea Buckthorn seed oil intake in obese children

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Background and aim: Childhood obesity is linked to early atherosclerosis. Monocytes generate a 'respiratory burst' of NADPH oxidase dependent superoxide anion production which is implicated in the pathogenesis of endothelial damage. Sea Buckthorn seed oil is rich in unsaturated fatty acids and antioxidant vitamins.

The aim of this study was to assess respiratory burst and other blood oxidative stress markers after 60 days of 800 mg/day Sea Buckthorn seed oil intake.

Materials and methods: Fortyone obese children (10–18 years old) referred for consultation at a general practitioner's in one year, were included in the study. Thirty controls were also involved. Chemiluminescence and spectrophotometric methods were used.

Results: Monocyte NADPH oxidase activity was correlated with plasma malonyldialdehyde level ($r = 0.33$, $p < 0.05$). In comparison to normal subjects, obese children had higher values for plasma ceruloplasmin ($p < 0.001$), apo B/apo A ratio ($p < 0.01$), blood pressure ($p < 0.001$), triglycerides ($p < 0.01$) and lower value for albumin/globulin ratio ($p < 0.01$), while CRP, fibrinogen, respiratory burst, lipid peroxidation measured as malonyldialdehyde and total antioxidant plasma capacity were increased but without statistical significance. After sea buckthorn seed oil intake, decreased values for respiratory burst ($p < 0.01$), ceruloplasmin ($p < 0.001$), triglycerides ($p < 0.04$), blood pressure ($p < 0.001$), and increased values for albumin($p < 0.03$), and albumin/globulin ratio ($p < 0.04$) were obtained.

Conclusions: Sea buckthorn seed oil intake decreases systemic oxidative stress by reducing monocyte NADPH oxidase activity. Also, by lowering blood pressure, triglycerides level and improving liver capacity for albumin synthesis, this treatment delays atherosclerosis in obese children.

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P18-3
Inhibition types and some kinetic properties of tyrosinase of deepwater pink shrimp (*Parapenaeus longirostris*) from edremit bay

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Phenoloxidases (POs) are a family of enzymes including tyrosinases, catecholases and laccases, which play an important role in immune defence mechanisms in various invertebrates. The aim of this study was to identify the tyrosinase activity present in the extract of deepwater pink shrimp (*Parapenaeus longirostris* (Lucas, 1846)) at Edremit Bay, (Eaegen Sea) by using as a substrate (L-Dopa) and different tyrosinase inhibitors(gallic acid and L-cisteine). Further characterisation was carried out in extracts partly purified using 40–70% ammonium sulfate fractionation. The enzyme had the highest activity at pH 4.0, and the optimum temperature values of shrimp tyrosinase for L-Dopa found to be 35°C. The inhibitory effect of gallic acid and L-cysteine on tyrosinase from deepwater pink shrimp were investigated. Gallic acid and L-cysteine inhibited the oxidation of 3-(3,4-dihydroxylphenyl)-L-alanine (L-DOPA) catalyzed by deepwater pink shrimp tyrosinase. Gallic acid showed mixed type inhibition with Ki and Ki' values of 8 and 2 mM respectively. L-Cisteine compound showed uncompatitive inhibition with Ki' value of 5.3 mM.

P18-4
Evaluation of the subcronic oral toxicity of a hight dose of maslinic acid in mice

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Maslinic acid is a pentacyclic triterpene found in olives and virgin olive oil. This minor component of *Olea Europaea* has been reported to have anti-carcinogenic, antidiabetic and antioxidant activities. The aim of this study was to evaluate the effect of the repeated oral administration of 50 mg/kg of maslinic acid in male Swiss CD1 mice during 28 days. Adult mice were randomly divided in two groups, a maslinic group ($n = 10$) that was administrated with 50 mg/kg and a control group ($n = 6$) that only received the solvent. Body weight was recorded daily while food and water consumption was controlled every 48 hour. At

the end of the study, blood was collected by cardiac puncture and was used for haematological and clinical chemistry analysis. Moreover, the vital organs were excised and its wet weight recorded. For the histopathological study the organs were embedded in paraffin, sectioned in 5 μm slices and stained with hematoxylin and eosin. No mortality was observed during the experimental period. The body weight was not affected by the administration of maslinic acid. Feed conversion efficiency (g gain/g feed) remained constant with no significant differences between groups. Haematological and clinical chemistry variables were not affected by the treatment. In the gross necropsy, no differences in the relative weight of spleen, liver, kidney, testicle, heart, lung and thyroid gland were observed. Histopathologic examination of these organs obtained at autopsy did not reveal any alterations. The results indicate the absence of adverse effects after repeated consumption of maslinic acid, thus constituting a first step in elucidating its future use as a nutraceutical.

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P18-5
Pharmacokinetic study of maslinic acid after oral administration to rats

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Maslinic acid is a minor non-nutritive component found in olives. This pentacyclic triterpene has been reported to exert beneficial effects on health. The aim of the present study is to determine plasmatic concentrations of maslinic acid in rat plasma after the administration of a single oral dose of 50 mg/kg. Maslinic acid was dissolved in 40% hydroxypropyl-beta-cyclodextrin and 0.5% sodium carboxymethyl cellulose and administered by gavage to 6-hour fasted rats. Blood was collected from the saphenous vein at 5, 10, 15, 20, 25, 30, 45 and 60 min. Plasma was obtained by centrifugation and samples were extracted with ethyl acetate followed by evaporation to dryness of the organic fraction. The residue was reconstituted with methanol-water (75:25 v/v). Analysis by HPLC with diode array-UV detection was carried out using a C18 column and mobile phase consisting of acetonitrile and water-acetic acid 0.05% v/v at 1 ml/min. The time course of maslinic acid concentration in plasma was analyzed by WinNonlin software. The pharmacokinetics of the triterpene was best described by a one-compartment, first order model and the estimated parameters were: AUC of 464.9 μmol/min/l, C_{max} of 3.74 μM, T_{max} of 18.0 min, V/F of 23.8 l/kg, Cl/F of 0.23 l/(min/kg), k_{01} of 0.17/min, k_{10} of 0.01/min. These results suggest that both intestinal absorption and elimination of maslinic acid take place by slow processes and are maintained over an extended time. In summary, this study constitutes the basis to further research on the formation of metabolites and tissue distribution of maslinic acid to provide complete knowledge of its pharmacokinetics.

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P18-6
Apolipoprotein A-I modulates processes associated with diet-induced nonalcoholic fatty liver disease in mice

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Apolipoprotein A-I (apoA-I) is the main protein of HDL. Here we investigated its involvement in diet-induced accumulation of triglycerides in hepatocytes and its potential role in the treatment of nonalcoholic fatty liver disease (NAFLD). ApoA-I deficient (apoA-$I^{-/-}$) mice showed increased diet-induced hepatic triglyceride deposition and disturbed hepatic histology while they exhibited reduced glucose tolerance and insulin sensitivity. Quantification of FASN-1, DGAT-1, and PPARγmRNA expression suggested that the increased hepatic triglyceride content of the apoA-$I^{-/-}$ mice was not due to *de novo* synthesis of triglycerides. Similarly, metabolic profiling did not reveal differences in the energy expenditure between the two mouse groups. However, apoA-$I^{-/-}$ mice exhibited enhanced intestinal absorption of dietary triglycerides (3.6 ± 0.5 mg/dl/min for apoA-$I^{-/-}$ versus 2.0 ± 0.7 mg/dl/min for C57BL/6 mice, $p < 0.05$), accelerated clearance of postprandial triglycerides, and a reduced rate of hepatic very low density lipoprotein triglyceride secretion (9.8 ± 1.1 mg/dl/min for apoA-$I^{-/-}$ versus 12.5 ± 1.3 mg/dl/min for C57BL/6 mice, $p < 0.05$). In agreement with these findings, adenovirus-mediated gene transfer of apoA-I_{Milano} in apoA-$I^{-/-}$ mice fed western-type diet for 12 weeks resulted in a significant reduction in hepatic triglyceride content and an improvement of hepatic histology and architecture. Our data show that in addition to its well-established properties on atheroprotection, apoA-I is also an important modulator of processes associated with diet-induced hepatic lipid deposition and NAFLD development in mice. Our findings raise the interesting possibility that expression of therapeutic forms of apoA-I by gene therapy approaches may have a beneficial effect on NAFLD.

P18-7
Changes in hepatic levels of PPAR-α, ACOX and CAT-1 in the prevention of hight fat diet iinduced steatosis

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The nutritional imbalance of a diet high in saturated fat and carbohydrates causes insulin resistance, inflammation and liver steatosis. N-3 fatty acids (EPA and DHA) increase insulin sensitivity, have anti-inflammatory properties, stimulate lipolysis and inhibit hepatic lipogenesis. PPAR-α is a transcription factor that is stimulated by n-3 fatty acids and regulates the expression of lipolytic proteins ACOX and CAT-1. This work evaluates the changes in ACOX, CAT-1 and PPAR-α levels in the prevention of high fat diet-induced steatosis. Male C57BL/6J mice ($n = 9$ for each group) were fed for 12 weeks with the following diets: (i) control diet (20% protein, 70% carbohydrate, 10% fat), (ii) control diet plus EPA + DHA (200 mg/kg), (iii) high fat diet (20% protein, 20% carbohydrate, 60% fat) or (iv) high fat diet plus EPA and DHA. Liver steatosis (histology), visceral fat, insulin resistance (HOMA), ACOX and CAT-1 (western blot) and PPAR-α (RT-

PCR) levels were analyzed. Group (iii) presented significant increases in visceral fat content, hepatic steatosis and HOMA and decreased significantly the levels of ACOX, CAT-1 and PPAR-α compared to controls (group i and ii). These effects were prevented in group (iv). Conclusion: In the prevention of high fat diet-induced steatosis there was observed an increment in PPAR-α, ACOX and CAT-1 levels. This suggests that they participate in the prevention of steatosis induced by high fat diet in mice. Supported by FONDECYT 1110043.

P18-8
Early effects of fish oil dietary intake on trout serum lipoproteins and muscle cholesterol deposition

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Fish oil enhances trout pigmentation and it also decreases incidence of cardiovascular diseases. However, there are contradictory studies about the effects of fish oil omega-3 on cardiovascular diseases. The effects of two different fish oil diets have been assessed, first in muscle pigmentation and them on serum lipoproteins and cholesterol muscle deposition. Twenty immature rainbow trout (mean weight 300 g) in duplicate were fed *ad libitum* during 8 days with diets containing 7% or 24% fish oil content respectively. By enhancing the amount of fish oil diet, a 30% increase in serum cholesterol and a 23% in muscle havee been obtained. On the other hand, serum triglycerides have rised spectacularly (127%). The augment of fish oil intake also produced a decrease in circulating serum carotenoids because they were easily transported into the muscle, obtaining a 54% increase in pigmentation due to a growth in recirculating lipoproteins. Finally, the infrared spectra showed a similar spectrum for both types of lipoproteins and so we can propose trout as model for the study of lipoprotein metabolism and atherosclerosis as well.

P18-9
Microbiological analysis of flour and whole-grain bread and biochemical indentification of bacterial isolates

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Flour and whole-grain bread were analyzed for total aerobic bacteria content using the most probable number method. Four samples – white bread, brown bread, wheat-groats bread and wheat-grain bread were quantitatively and qualitatively examined. While the white and brown breads which were produced from flourcontain no preservatives and GMO the wheat-groatsand wheat-grain breads, produced from whole grains, contain additives as antioxidants, emulgators and E 282- calcium propionate as mould inhibitor. Our results showed that 1 cm^3 of each examined bread sample contain the following total number of aerobic bacteria: 1.8×10^6 – in the white bread, 9.0×10^6 – in the brown bread, 8.9×10^4 – in the wheat-groats bread and 7.6×10^3 – in the wheat-grain bread. The probable reason for the higher number of bacteria in the white and brown bread is the lack of preservatives. Twenty bacterial isolates were cultured andtheir morphology was microscopically analyzed. Bacteria obtained are motile cocci as well as short and long sized rods. Additionally Gram staining and spore analysis was done and the biochemical indentification of the isolates was performed *via* Polimicrotest.

P18-10
Effect of proinflammatory cytokine TNF-α on Caveolin-1 gene methylation during adipocyte differentiation. Relation to insulin signalling

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Obesity is a metabolic disease, arising as a consequence of the interaction between environmental and genetic factors, in which regulation of gene expression by epigenetic mechanisms has a critical role. A low degree inflammation mediated by cytokines such as TNF-α is also associated with this disorder. Enlargement of fat depots by adipogenesis is also a main feature of obesity, and Caveolin-1, highly expressed in adipocytes, has a key role in the structural and functional organization of insulin signalling. Since Caveolin-1 (Cav-1) has been shown to be regulated by methylation of its promoter, we decided to study the importance of this epigenetic regulation during adipogenic differentiation.

We have determined changes in the methylation pattern of Cav-1 gene during adipocytic differentiation in the presence of TNF-α and how they affect Cav-1 expression and subsequently insulin signalling. Mouse 3T3-L1 cells were differentiated following the classical protocol during 21 days in the presence of 10 ng/ml of TNF-α, taking samples at 0, 7 and 21 days. The region of Cav-1 gene studied (from 619 bp 5′ to 1333 bp 3′ of the ATG codon) includes two CpG islands located in the proximal promoter and the first intron. Methylation percentage of CpG dinucleotides were determined by MassArray Epityper. Cav-1, Insulin receptor (IR), PKB/AKT and GLUT-4 expression was measured at mRNA and protein level by real time RT-PCR and western blot, respectively. Activation of these proteins was also determined by western blot using anti-phosphoprotein antibodies.

A significant increase in methylation of both CpG islands was observed in the presence TNF-α during the adipogenic differentiation. This higher methylated state seems to be accompanied with a significant reduction in Caveolin-1 expression (mRNA and protein) which translates into changes in the insulin signalling pathway intermediaries measured.

P18-11
Anti-inflammatory effects of nucleosides in human intestinal cells

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Nutritional therapy for inflammatory bowel diseases is gaining interest. Dietary nucleosides, in addition to the effect on the nutritional status, may exert a therapeutic effect due to their important role in cellular and humoral immunity, intestinal growth, differentiation and recovery from tissue damage. In addition, nucleosides modulate many metabolic processes directly or through their effect on gene expression and are considered as nutriceuticals.

The aim of this study was to evaluate the effect of exogenous nucleosides. In particular, we focused on expression of proteins

implicated in the inflammatory processes, such as interleukins (ILs), cytokines and proteins related to the extracellular matrix. For this purpose, we induced inflammation by adding IL-β1 to epithelial human Caco2 cells originating from colorectal adenocarcinoma, which is one of the most affected regions of the intestine in inflammatory bowel diseases. The effect of intestinal inflammation on cytokine, MMP1, fibronectin and collagen expression and release was determined using a combination of Western blots, RT-PCR and ELISA.

As expected, the expression of cytokines increased by challenging Caco2 cells with the inflammatory inductor. Addition of exogenous nucleosides to the culture media resulted in a general reduction of cytokine mRNA content and protein secretion, and an increase in collagen mRNA and protein content in the inflamed cells. Interestingly, the effect of exogenous nucleosides depended on the presence or absence of inflammation.The anti-inflammatory effect is clear, whereas in normal conditions they appear to increase some of the markers, suggesting that there is more than one mechanism of action involved.

P18r-12
Docosahexaenoic acid (DHA) and procyanidins dimers (B_1 and B_2) and trimer (C_1) are strong and selective inhibitors of Cyclooxygenase-1 and 2

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The metabolic and immune systems are among the most fundamental requirements for survival; moreover, the inflammatory response has been implicated in the pathogenesis of many chronic diseases. Along these lines, the modulation of inflammation by consuming bioactive food compounds, such as ω-3 fatty acids or procyanidins, is a powerful tool to promote good health. Prostaglandin endoperoxide H synthases-1 and 2 (PGHS-1 and PGHS-2), or cyclooxygenases-1 and 2 (COX-1 and COX-2), catalyze the committed step during the metabolic conversion of ω-6 arachidonic acid (AA), the most abundant fatty acid present in the cell membrane, into eicosanoids. Eicosanoids act as chemical messengers in the immune system, and their main function involves regulating the inflammatory response.

Using a cell-free assay we have elucidated the kinetic relationships between docosahexaenoic acid (DHA) and procyanidins (B_1, B_2 and C_1) with both constitutive and LPS-induced cyclooxygenase isoforms (COX-1 and COX-2 respectively). In this way, the fifty percent inhibitory concentration (IC_{50}) values obtained show the discriminatory capacity of DHA and pure procyanidins versus the inhibition the two cyclooxygenases activities, being DHA and B_1 strong and selective inhibitors of COX-1 activity, whereas B_2 and C_1 are selective inhibitors of COX-2 activity at the used concentrations. DHA and pure procyanidins are able to alter the substrate specificity of COX-1 and COX-2 to AA, as the result of the modulation of the maximal reaction rate (V_{max}) and the Michaelis-Menten constant (K_m). DHA exhibited mixed inhibition behaviour towards COX-1, whereas B_1, B_2 and C_1 seem to compete with AA in binding to the active site of the COX-1 and COX-2 respectively. This strong and selective inhibition of cyclooxygenase activities was supported by the inhibition of lipopolysaccharide (LPS) induced prostaglandin E_2 (PGE_2) secretion in primary human monocytes pre-treated with physiologic concentrations of DHA and procyanidins.

Therefore, the selective inhibition of COX activity by food bioactive elements, such as DHA and procyanidins B_1, B_2 and C_1, lead to great interest in their use as potential anti-inflammatory compounds, as well as the inhibition of PGE_2 secretion in primary human monocytes is a relevant proof of the capacity of bioactive food compounds to promote good health through the modulation of inflammation with diet.

P18-13
Grape-seed derived procyanidins decrease intestinal dipetidyl-peptidase 4 (DPP4) activity and improve glycaemia

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The inhibition of the enzyme dipeptidyl-peptidase 4 (DPP4) is currently assayed as a mechanism to improve glycaemic control in the treatment of diabetes mellitus type 2. We had previously shown that procyanidins derived from grape seed (GSPE), which ameliorate some hyperglycaemic situations, inhibit DPP4 activity. In the present study we evaluate the acute effects of GSPE on intestinal DPP4 activity and the possible incretin effect.

In vivo effects of an acute GSPE treatment (1 g GSPE/kg bw) for 1 hour on intestinal and plasmatic DPP4 activity were analysed on Wistar rats. Rats treated with 1 mg Vildagliptin/kg bw for 1 hour were used as positive control. We measured DPP4 activity by colorimetric determination of product (H-Gly-Pro-pNA, 0.2 mM, Bachem) and glucose concentrations were assayed using and enzymatic colorimetric kit (GOD-PAD method from QCA). Intestinal DPP4 activity was inhibited in rats treated with GSPE but no effects were found in DPP4 plasma activity. The effects of that treatment on glucose levels were evaluated after a glucose load (2 g Glucose/kg bw for 20 min). We found a lower increase on plasma glucose after GSPE treatment, a similar pattern than that of Vildagliptin.

In conclusion, an acute dose of GSPE can limit the acute glucose load suggesting an incretin effect. These effects could be related to the inhibition of intestinal DPP4 activity.

P18-14
Effects of D-fagomine on metabolic alterations in rats fed an energy-dense diet

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Insulin resistance (IR) and obesity are risk factors for type-2 diabetes and cardiovascular disease that are related to the glucose metabolism. IR may be triggered by an excess of energy-dense food and has been related to the accumulation of fat in different organs. We have shown that the iminocyclitol D-fagomine (a D-glucose analogue first isolated from buckwheat seed) lowers postprandial plasma glucose levels without stimulating insulin secretion. With the aim of evaluating the long-term effects of D-fagomine on the metabolic alterations induced by an energy-dense diet, we divided 15 Sprague–Dawley rats (aged 28 weeks) into three groups and fed them a standard diet, a high-fat and high-sucrose diet (HF-HS) and HF-HS diet supplemented with D-fagomine (0.65 g/kg feed, HF-HS-FG) for 24 weeks. Feed consumption; weight gain; blood pressure; plasma glucose, insulin, ghrelin, leptin, glucagon and adiponectin; and urine F2-isoprostane were monitored via measurements at different times. At the

end of the experiment, body fat accumulation and liver fat deposits were examined. Plasma levels of glucose and insulin in the control group and the HF-HS-FG group were similar, while insulin concentration in the HF-HS group was four-fold higher. The levels of glucagon, ghrelin and F2-isoprostane in the rats supplemented with D-fagomine were lower than in the HF-HS group. Liver weight was normal in the D-fagomine group and histology showed that D-fagomine moderated the liver inflammation and steatosis triggered by the HF-HS diet. In conclusion, D-fagomine reduces the incidence of an energy-dense diet in plasma insulin levels, liver steatosis and liver inflammation in rats. These effects are probably related.

P18-15
Epigenetic effects of diet: microRNAs and dietary flavonoids

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Epigenetic modifications may be one mechanism by which exposure to nutritional factors influence gene expression and modulate the phenotype. In this way, diet could modulate the development of chronic diseases, such as cardiovascular diseases, dyslipidaemia, obesity, insulin resistance and type 2 diabetes. The epigenetic mechanisms described as regulated by nutritional factors include DNA methylation, histone modifications and noncoding microRNAs (miRNA).

MicroRNAs (miRNAs) are small non-coding RNAs of 18–25 nucleotides in length that bind to complementary 3′UTR regions of target mRNAs, inducing the degradation or transcriptional repression of the target. A single miRNA can regulate the expression of multiple target mRNAs, and each mRNA may be regulated by more than one miRNA. miRNAs have been reported to regulate several metabolic pathways such as insulin secretion, carbohydrate and lipid metabolism. Furthermore, miRNAs have been shown to be related to several human diseases and their modulation can provide therapeutic benefits.

Interestingly, dietary factors, including micronutrients and non-nutrient dietary components, have been shown to alter miRNA gene expression. For instance, dietary polyphenols such as soy isoflavones, epigallocatechin gallate and proanthocyanidins modulate miRNA expression.

Grape seed proanthocyanidins rapidly repress the expression of miR-33 and miR- 122 in rat hepatocytes *in vivo* and *in vitro*. Furthermore, the miR-33 target gene ATP-binding cassette A1 (Abca1) and the miR-122 target gene fatty acid synthase (Fas) are also modulated by proanthocyanidins. These results highlight a new mechanism by which grape seed proanthocyanidins produce hypolipidemia through their effects on miRNA modulators of lipid metabolism.

P18-16
Proanthocyanidins modulate miR-33a and its target gene ATP-binding cassette transporter A1 in HepG2 cells

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Hypercholesterolemia is an independent risk factor for cardiovascular disease and atherosclerosis that are two of the major health problems in western countries. Proanthocyanidins are the most abundant polyphenols in human diets, mainly provided by vegetables, cacao and red wine. Recently, it has been described that a grape seed procyanidins extract (GSPE) modulate the expression of several microRNAs. microRNAs are small non-coding RNA of approximately 19–24 nucleotides in length that regulate the expression of target genes at post-transcriptional level. Specifically, miR-33a/b has recently been identified to be potent regulator of cholesterol metabolism genes including the ATP-binding cassette transporter A1 (ABCA1). In liver, ABCA1 facilitates the efflux of phospholipids and cholesterol to apoA-1 to generate nascent HDL particles, which promote the reverse cholesterol transport and prevent the accumulation of lipids in the artery wall. The aim of this study was to evaluate whether proanthocyanidins modulates liver ABCA expression through the regulation of miR-33a in human hepatocarcinoma cell line, HepG2. To this end, HepG2 cells were treated with 50 mg GSPE/L, after 8 hours of treatment total RNA and proteins were extracted. Results showed that GSPE treatment reduces SREBP2 expression by 39%. Similarly, the expression of miR-33a was decreased by 44%. Moreover, we showed that ABCA1 mRNA and protein levels were increased after 8 hour of treatment. In conclusion, proanthocyanidins modulate the expression of miR-33a and its host gene, SREBP2, leading to an increase in ABCA1 expression. Further studies are needed to elucidate if miR-33b is also modulated by proanthocyanidins and to understand the mechanism trough which proanthocyanidins modulate miR-33a/b expression.

P18-17
The effect of lipoic acid and diallyl trisulfide on the activity of aldehyde dehydrogenase and rhodanese

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Our attention was drawn to the studies linking thiol compounds: lipoic acid (LA) and diallyl trisulfide (DATS) with aldehyde dehydrogenase (ALDH) and rhodanese activities. LA is a naturally occurring thiol compound that acts as a coenzyme of multienzymatic mitochondrial complexes catalyzing oxidative decarboxylation of α-ketoacids. Broccoli and spinach are good dietary sources of LA. DATS is a garlic-derived sulfur compound.

Both compounds have a well-documented biological activity. LA has beneficial therapeutic effect in diabetes, degenerative processes and immune deficiency syndrome. Garlic-derived DATS has received increasing interest due to its potential therapeutic efficacy in cardiovascular and cancer diseases.

ALDH is an important enzyme that eliminates toxic aldehydes by catalyzing their oxidation to non-reactive acids. Rhodanese plays a key role in the protection from cyanide poisoning. It is responsible for transfer of highly reactive sulfane sulfur from thiosulfate to cyanide (or other acceptors).

Our studies performed *in vivo* documented that ALDH activity in the liver of LA-treated (*ip*) rats was significantly decreased. Additionally, the *in vitro* studies confirmed an inhibiting effect of LA on the ALDH activity. Our pilot study has suggested that LA is a competitive inhibitor of ALDH.

Other experiments revealed a significant increase in rhodanese activity in the liver of rats previously treated *ip* with LA.

The studies conducted *in vivo* on mouse livers demonstrated that DATS increased the activity of rhodanese and did not influence ALDH activity.

To conclude, our studies can shed a new light on the role of aldehyde dehydrogenase and rhodanese in the development of many diseases and on their treatment since LA and DATS modulate the activity of both enzymes. These thiol compounds may be used as dietary supplements, and due to their effect on the activity of ALDH and rhodanese, they offer fresh opportunities for the potential therapeutic use.

P18-18
Extracts of *Rubus fruticosus* decrease fat depots and improve glucose/insulin relationship in female rats

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Obesity and diabetes have reached epidemic proportions throughout the World. Berry fruits are rich sources of antioxidant phenolics which may protect against degenerative diseases. In order of determining how a diet supplemented with a concentrated extract of *Rubus fruticosus* (RF) could affect adipose tissue mass and insulin sensitivity, Sprague Dawley female rats weighing around 100 g were divided into three groups. These were fed *ad libitum* with the standard pellet diet (Controls, C), a cafeteria diet (pellet, sugar, condensed milk, croissants, muffins and butter) (CD) or the CD supplemented with *RF* (corresponding to 40 mg anthocyanins/100 g) (CD-R). The standard diet had 310 Kcal/100 g, whereas both CD and CD-R had 474 Kcal/100 g. Food intake did not differ between groups. At 50 days on the diets both CD and CD-R groups had higher body wt. than C and an index of insulin sensitivity (QUICKI) was lower in CD that in the other two groups, whereas plasma triacylglycerols were lower in CD-R than in CD. At 90 days of treatment, rats were subjected to an oral glucose tolerance test (OGTT, 2 g glucose/kg) and whereas the area under the curve (AUC) of glucose did not differ between the groups, the AUC for insulin was higher in CD versus C, while it was lower in CD-R than in CD, without differences between C and CD-R. After the OGTT rats were sacrificed and total body weight as well as most adipose tissues (inguinal, perirenal, mesenteric and lumbar) and liver weight in CD was higher than in C, whereas in CD-R values were closer to those of C. Thus, long-tem dietary supplement with RF extract in female rats fed cafeteria diet was able to decrease body weight gain and body fat depots and to improve glucose/insulin relationships.

P18-19
Effect of daily oral administration of *trans*-Resveratrol for 28 days on antioxidant status of testes in healthy rats

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trans-Resveratrol is a polyphenol which has been shown to enhanced spermatogenesis. Moreover, this polyphenol is also considered a potent antioxidant. Since oxidative stress is one of the major causes of male infertility, the aim of the study was to assess the antioxidant protection that *trans*-resveratrol can exert in testes. Male Sprague–Dawley rats (n = 6–7/group) were divided in three groups. A daily oral administration by gavage of *trans*-resveratrol dissolved in 20% cyclodextrin was given for 28 days to two groups at the doses of 20 (RSV 20) and 60 (RSV 60) mg/kg, respectively. A third group received only the solvent which was considered as a control. At the end of the experiment, testes were removed, rinsed with PBS, frozen with liquid nitrogen and kept at -80°C. Testes were divided into pieces to carry out 20% (w/v) homogenates with different buffers and aliquots of supernatants were used for the determination of superoxide dismutase (SOD), catalase (CAT) and thioredoxin reductase (TrxR) activities and oxidized/reduced glutathione ratio (GSH/GSSG) to assess the antioxidant status of the organ. Results showed that the administration of *trans*-resveratrol decreased SOD activity in a 52% (44.3 ± 9.4 U/mg protein) and 80% (18.7 ± 5.5 U/mg protein) in RSV 20 and RSV 60 groups, respectively. SOD activity in Control group was 92.1 ± 18.9 U/mg protein. However, *trans*-resveratrol treatment affected neither CAT and TrxR activities nor GSH/GSSG ratio. In conclusion, the effect of *trans*-resveratrol observed in spermatogenesis may be explained, at least in part, to the reduction of SOD activity in testes.

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P18-20
Non-covalent interactions between dietary polyphenols and bovine β-lactoglobulin: Effect on the protein structure, digestibility and total antioxidant capacity

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In solution, polyphenols can form non-covalent interactions with globular proteins resulting in complexation, stabilization of protein structure, protein unfolding and precipitation. We have tested how changes in pH that occur in the gastrointestinal tract (GIT) (from 1.2 to 2.5 in the stomach to 7.4 in the intestine) affect binding affinity of polyphenolic compounds to a major whey protein, β-lactoglobulin (BLG), and whether non-covalent protein-polyphenol interactions influence protein digestion and radical scavenging activity of dietary polyphenols.

The interactions between BLG and polyphenols extracted from teas, coffee and cocoa were studied by fluorescence and CD spectroscopy. The influence of polyphenols on the faith of the protein in the GIT was assayed by *in vitro* pepsin and pancreatin digestion. The effect of complexation on the antioxidant activity of polyphenols was studied by ABTS assay.

Polyphenols of all tested common beverages made weak non-covalent interactions with surface residues of BLG at both acidic and neutral conditions and the strength of interaction varied with pH. The most profound effect of pH on binding affinity was observed for coffee polyphenol extract (PE). All tested PEs protected protein secondary structure at extremely acidic pH of 1.2. Coffee and cocoa PE delayed pepsin and pancreatin digestion of BLG and induced β-sheet to α-helix transition at pH 7.2. Antioxidant activities of all components, protein and polyphenols, were influenced by the non-covalent binding resulting in reduced radical scavenging activity of the food matrix. A good correlation was observed between the strength of PE-BLG interactions and (i) half time of BLG decay in gastric conditions ($R^2 = 0.92$), (ii) masking of total antioxidant capacity ($R^2 = 0.96$).

The polyphenol- BLG systems are stable at acidic and neutral pH values providing both protein and polyphenolic compounds pro-longed life in the GIT. BLG could be a potential carrier of various polyphenols.

P18-21
Gestational programming of impaired brown adipose tissue innervation and obesity in rats is offset only in females by differential innervation of white adipose tissue

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We recently described that excessive fat accumulation in adult male offspring of calorie-restricted pregnant rats may be associated with reduced sympathetic innervation in the inguinal adipose tissue (iWAT), leading to hyperplasia. Here, we aimed to assess whether brown adipose tissue (BAT) is also less innervated as a consequence of this prenatal imprinted-condition and whether these alterations in iWAT and BAT could affect lipid metabolism and energy balance regulation capacity. Offspring of control and 20% calorie-restricted rats (days 1–12 of pregnancy) (CR) were studied at the age of 25-days. Body weight and iWAT and BAT weights were recorded. Protein levels of TH (iWAT and BAT) and UCP1 (BAT) and mRNA expression of lipid metabolism-related genes (iWAT and BAT) were analyzed. No differences were found in body weight or iWAT and BAT weights between control and CR. In iWAT, CR males, but not females, displayed lower TH levels than controls, in accordance with the described lower sympathetic innervation. This was accompanied by sex-dependent changes in mRNA levels of ATGL, LPL and CPT1, with a tendency to lower levels in males and higher levels in females. In BAT, both genders of CR displayed lower TH and UCP1 levels than controls, as well as lower LPL and CPT1 mRNA levels. These results suggest that adipose tissue sympathetic innervation is impaired in CR animals, but in a sex- and tissue-dependent manner, and may be responsible for the detrimental effects of gestational caloric restriction on adult body weight and related metabolic alterations, and is more exacerbated in males.

P18-22
Genetically engineered multivitamin corn feeding assessment

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Our genetically engineered (GE) multivitamin corn produces high levels of beta-carotene, lutein, zeaxanthin, lycopene, ascorbate and folate. This makes it an antioxidant crop which can potentially address micronutrient deficiencies caused by lack of vitamins A, B9 and C in developing countries and it might play a role in prevention and progression of several diseases. As a GE crop, it must be assessed to evaluate any effects on human and animal health and the environment as an integral part of the approval process before entering the market. The work is focused on the assessment of toxicity and preliminary allergenicity of this multivitamin plant. Bioinformatics analysis was performed to compare the expressed recombinant protein sequences to known allergens. No allergenicity or allergic cross-reactivity was identified. Sub-acute and sub-chronic toxicity feeding trials were performed. Mice fed with diets enriched with whole GE corn and control diets (conventional corn counterpart and standard mice diet) were carried out to study potential sub-acute and sub-chronic toxicity for 28 and 90 days, respectively. Based on a comparative approach between animals fed with a GE corn based diet and animals fed with control diets, statistical methods were employed to analyze food consumption, body weight, hematological and biochemical blood parameters, organ weight and histopathology. The statistical results showed no differences between the multivitamin corn fed group and control groups and histopathological analysis revealed no differences in terms of hepatocellular, myocardial, adrenal gland and renal tubular hypertrophy, neurotoxicity in the brain and toxicity-related alterations in reproductive or lymphoid organs. The 28-day sub-acute and 90-day sub-chronic toxicity feeding studies indicate that the GE corn does not induce any toxicity and it does not have any potential allergenicity.

P18r-23
Different health outcomes of prenatal and postnatal nutritional conditions affecting obesity susceptibility may be related with different programming effects on similar metabolic targets

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Maternal nutrition during perinatal life may affect the predisposition of offspring to develop obesity in later life. Here, our aim was to assess whether early programming of obesity susceptibility due to maternal caloric restriction during pregnancy or lactation may be related with differential programming effects of energy metabolism related genes in different tissues. For this purpose, offspring of two different models of perinatal nutrition, with different outcomes were studied: (i) 20% maternal caloric restriction during the first half of gestation, and (ii) 20% maternal caloric restriction during lactation. At weaning, part of the pups were sacrificed and the retroperitoneal white adipose tissue (WAT), liver and interscapular brown adipose tissue (BAT) were collected to perform analysis. Remaining animals were kept alive on a normal-fat-diet and followed until 4 months. At 4 months of age, male and female offspring of caloric-restricted dams during gestation (CRG) showed higher food intake than controls, but only males displayed higher body weight. However, at weaning no differences were found in body weight or weight of WAT, liver and BAT between control and CRG animals. At this early life, interestingly, CRG rats showed lower mRNA expression levels of InsR (WAT and liver), ATGL (WAT-only males- and liver), UCP2 (WAT- only males), and CPT1 (BAT). CRG rats also displayed lower UCP1 protein levels than controls. In contrast, both male and female offspring of calorie-restricted lactating dams (CRL) showed lower body weight and food intake than their controls during the whole follow-up. CRL pups also displayed, at weaning, lower weights of WAT, liver and BAT than controls. Moreover, CRL animals showed higher mRNA levels of ATGL (WAT and liver), InsR and IRS1 (WAT), ObRb (liver), CPT1 (liver and BAT) and UCP1 (BAT). In conclusion, nutritional conditions during perinatal life result in early adaptations affecting the metabolic capacity of different tissues, which may be related with the different propensity to obesity development in adulthood.

P18-24
Protective effects of melanoidins derived from dried apricots on oxidative damage and mitochondrial-dependent endothelial cell death

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The cardiovascular (CV) benefits associated to diets rich in fruit and vegetables are thought to be due to phytochemicals contained in fresh plant material. However, whether processed plant foods provide the same benefits as the unprocessed ones is an open question. During food transformations high molecular-weight polymers called melanoidins (MEL) are formed. We have previous reported that MEL obtained from prunes protect human endothelial cells (ECs) from hydrogen peroxide-induced oxidative stress. However, only partial structures of MEL have been elucidated so far, therefore MEL from different food may have different structures and activities. MEL from heat-processed apricots were isolated and their presence confirmed by browning index. Oxidative-mediated ECs injury is the key step for onset and progression of CV diseases, therefore the potential protective effect of apricot melanoidins on hydrogen peroxide-induced oxidative mitochondrial damage and cell death was explored on ECs. The redox state of cytoplasmic and mitochondrial compartments was detected by using the *redox-sensitive*, *fluorescent protein* (roGFP), while the mitochondrial membrane potential (MMP) was assessed with the fluorescent dye, JC-1. ECs exposure to increasing doses of hydrogen peroxide, dose-dependently induced mitochondrial and cytoplasmic oxidation. Further detected hydrogen peroxide-elicited phenomena were MMP dissipation and ECs death. Pretreatment of ECs with apricot melanoidins, significantly counteracted and ultimately abolished *hydrogen peroxide* elicited phenomena. Since MEL may reach *in vivo* concentrations comparable to the ones we used *in vitro*, we suggest our results could be representative of a physiologically relevant *in vivo* mechanism.

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P18r-25
Impact of early leucine supplementation on glucose-insulin homeostasis

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Introduction: Early nutrition plays a relevant role in development and may affect the susceptibility to future health outcomes.
Objective: The main aim of this study has been to assess the impact of maternal leucine (Leu) supplementation during lactation on the glucose-insulin homeostasis in adult offspring.
Methods: At 24 hour after parturition, Wistar rats were fed either with standard diet (controls, C) or with this diet supplemented with 2% Leu. Then, at weaning, Leu supplementation was removed to half of the animals (group L) or maintained (group L^+). At 5 months of age, insulin and glucose homeostasis was checked performing an oral glucose tolerance test (OGTT). At the 5.5 months age, animals were sacrificed and blood samples were collected.
Results: Both female and male L animals showed a normal response to OGTT. Interestingly, this was associated with higher insulin levels in females and lower in males (in comparison with the respective controls). As a result, the HOMA index in both sexes could be considered non different between C and L animals. However, when Leu supplementation was maintained all the way through adult life, females L^+ showed a lower glucemia induced by OGTT and the males no differences, versus the respective controls. Accordingly, females required less insulin and males more insulin (although in any case statistically significant differences were not attained) and at the end, HOMA index was no different between C and L^+ animals.
Conclusion: Increasing dietary recommendations of essential amino acids at early stages, including Leu, are being proposed. We have shown that a moderate increase in maternal dietary Leu during lactation has an impact in the handling of the glucose-insulin homeostasis in adult offspring, which shows a gender-specific modulation. In males, this would be beneficial whereas in females, the beneficial effect would be seen with prolongation of the treatment to adulthood.

P18-26
Metabolic programming of obesity

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The molecular basis explaining the system that controls body weight is a good example of biological systems complexity, which is very relevant, as obesity has become a major health and economic concern in our developed societies. Overweight, obesity and related medical complications can occur as a result of genetic or acquired changes. Epigenetic marks or imprinting, affect gene expression along life without changing the DNA sequence, and it is increasingly known how different food components act on specific targets in this system, which is influenced by a number of genetic variants in more than a hundred genes. Life style and its related metabolic history also affect the imprinting for predisposition to obesity and to develop the obesity-associated medical problems. Failures in imprinting cause extreme rare forms of obesity but, interestingly, environmental exposures during critical developmental periods appear to affect the profile of epigenetic marks and result in more frequent types of obesity.

The new role of a regulatory nutrient during lactation, leptin, as recently discovered in our laboratory is a good example and will be discussed. We knew from epidemiological data that breastfeeding compared with infant formula confers protection against obesity, insulin resistance and diabetes type 2, and other chronic diseases later in life. Our studies have identified leptin as the specific compound that is responsible for beneficial effects of breastfeeding. Direct cause-effect evidence was obtained in various long term studies in rats, where oral leptin supplementation (physiological doses) during the suckling period resulted in a decrease in food intake, affected food preferences, and protected against overweight in adulthood, with an improvement of parameters such as leptin and insulin sensitivity. Indirect evidence in humans have been obtained and mechanistic studies have shown the biological plausibility of the observed effects.

P18-27
Retinyl palmitate, but no β-carotene, supplementation during the suckling period affects adipose tissue development in young rats

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Despite the increasing evidence linking vitamin A to the control of adiposity in adult animals, little is known about its effects in early life. We aimed to assess the impact of a moderate supplementation with vitamin A in early life on white adipose tissue (WAT) development.

Rat pups received during the suckling period a daily oral dose of vitamin A corresponding to approximately three times the vitamin A ingested daily from maternal milk, in the form of retinyl palmitate (RE) or β-carotene (BC). Control rats received the vehicle (olive oil). The day after weaning, vehicle-, RE- and BC-treated rats were sacrificed and inguinal WAT morphology and gene expression was analyzed.

RE and BC-treated young rats showed no differences in body weight, adiposity or leptin levels in serum, but only RE-treated rats displayed a trend for higher percentage of smaller cells and gene expression features consistent with an increased cell proliferation potential in iWAT, which correlated with a reduced expression of adipogenic markers. Interestingly, only in the RE-treated animals, together with the typical proliferating precursor-cells, we observed proliferating cells with small cytoplasmic lipid droplets, i.e., presumably juvenile adipocytes. Evidence was obtained for increased retinoic acid-induced transcriptional responses in tissues of RE-treated neonatal rats, which expressed the retinoic acid synthesising enzyme Aldh1a1.

Our results suggest that vitamin A supplementation in the form of RE, but not BC, during the active phase of early postnatal WAT development may favour the development of a pool of immature adipocytes retaining a competent proliferative status. This could in turn condition long-term effects on body adiposity upon a subsequent dietary challenges, such as a high-fat diet.

P18-28
Administration of a probiotic *Escherichia coli* Nissle 1917 increases the bioavailability of amiodarone in male rat

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Escherichia coli is a bacterial strain occuring in the intestinal microflora. The bacteria of an *E. coli* strain can be pathogenic, non-pathogenic and even probiotic. The aim of this study was the find whether probiotic *E. coli* strain (*E. coli* Nissle 1917, serotype O6:K5:H1) can influence pharmacokinetics of concomitantly taken pharmacotherapeutic agent, antiarrhythmic drug amiodarone. The probiotic strain was administered to thirty male Wistar rats for seven days and the saline solution was administered to another thirty experimental animals for seven days as a control. On the eighth day, the amiodarone hydrochloride was applied to all sixty rats. After 1, 2, 3, 4, 5.5, 7, 9, 14, 22 and 30 hours the blood samples were taken from rat abdominal aorta; afterwards, the plasma level of amiodarone and of its metabolite N-desethylamiodarone was measured by HPLC-UV. Pharmacokinetic data of amiodarone were compared with amiodarone pharmacokinetics after application of a non-pathogenic and non-probiotic *E. coli* strain (ATCC 25922). The results show that the application of the reference ATCC 25922 strain does not influence the amiodarone pharmacokinetics; on the other hand, the application of probiotic *E. coli* Nissle 1917 causes increased bioavailability of amiodarone from the gastrointestinal tract. The level of metabolite is also increased after *E. coli* Nissle 1917 administration. This result is in line with increased activity of the liver CYP2C enzymes found in our earlier study in the presence of the probiotic [1] (CYP2C enzymes are known to take part in deethylation of amiodarone in the rat and human [2]). Taken together, the administration of *E. coli* Nissle 1917 (serotype O6:K5:H1) as a probiotic strain may exhibit its beneficial effect through better bioavailability of the drug.

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P18-29
Interaction of anthocyanidins with human liver microsomal cytochromes P450 1A2 and 3A4 *in vitro*

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Anthocyanidins, a class of flavonoids, are common plant red-blue pigments. They are the sugarless (aglycone) parts of anthocyanins. Anthocyanidins are found in many colored fruits and vegetables (e.g. blueberries, red grapes, cranberries, raspberries, blackberries, red cabbage, red onions, egg plant) and are most widely known for their antioxidant properties. Six most important anthocyanidins (cyanidin, delphinidin, pelargonidin, malvidin, peonidin and petunidin) were tested for their potential to inhibit the enzyme activities of human liver microsomal cytochromes P450 (CYP), especially CYP1A2 and CYP3A4 (ethoxyresorufin O-deethylase and testosterone 6β-hydroxylase). The results have shown inhibition of the activity of CYP1A2 (by delphinidin, down 70%) and of the CYP3A4 enzyme. The enzyme activity of CYP3A4 was influenced by all tested compounds; cyanidin, about 50%; delphinidin, about 90%; pelargonidin, about 70%; malvidin, about 65%; peonidin, about 80%; and petunidin, about 80% (the data correspond to the remaining activity of the CYP3A4 with 100 μM anthocyanidin concentration). The inhibition of CYP3A4 activity by anthocyanidins opens the possibility of drug interactions during a co-administration of other drugs metabolized by the same enzyme.

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P18-30
Anti-oxidant and anti-migration activity of supercritical carbon dioxide extracts of *Pistacia lentiscus* L. on human endothelial cells

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The regular consumption of fruit and vegetables is associated with a low incidence of cardiovascular events. Endothelial Cell (EC) injury, through oxidative stress, is a pivotal event in cardiovascular diseases (CVD) development, therefore a great deal of attention is now focused on natural antioxidants for both CVD prevention or treatment. In this work the anti-oxidant and anti-migration effect of a Supercritical Fluid Extract (SFE) obtained by *Pistacia lentiscus* L. (Pl) leaves was investigated in human ECs. The Pl plant is an aromatic bush indigenous to Italy and other Mediterranean and Middle East countries. Its leaves extract have found extensive use in folk medicine for several therapeutic uses including the anti-hypertensive, the anti-inflammatory and the antiseptic. Extraction by means of supercritical carbon dioxide was preferred to traditional techniques because of the obtaining of pure extracts deprived of organic solvents and of degradation compounds; moreover the extract is not modified by the loss of water-soluble compounds. The Pl extract, tested in a cultured ECs model attenuated the serum-induced cellular migration at the concentration 200 μg/ml without affecting their proliferation. The Pl extract also inhibited serum- and hydrogen peroxide-elicited reactive oxygen species (ROS) production in ECs, suggesting the PL's antioxidant property as a part of the mechanism by which ECs migration is being inhibited. Although further studies *in vivo* are necessary to attribute a potential therapeutic role of Pl extract/s in the treatment of CVD, this is the first study reporting its anti-oxidant and anti-migration effect on a human vascular model. Acknowledgments: Study funded by the Sardinia Region through Sardegna Ricerche Technology Park. Partial support also came from Banco di Sardegna Foundation and L.R. 7/2007 call 2008. AMP was supported by Ricerca cofinanziata PROGRAMMA OPERATIVO FSE SARDEGNA 2007–2013-L.R.7/2007-Promozione della ricerca scientifica e dell'innovazione tecnologica in Sardegna.

P18-31
Antioxidant effects of Cagnulari grape marc extracts on two human endothelial cell models

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Although wine production is one of the most important agricultural activities worldwide, the problems related to its waste treatment or disposal are far from being resolved. Wine industry wastes may account for about 30% of the grapes used for wine production, which by-products may bear a considerable burden of phenolic components depending on the type of grape, the part of the tissue and the processing conditions. It appear therefore evident that the wine waste loads can be significantly reduced through the use of new or modified processing methods aimed at obtaining added-value bio-products, such as natural antioxidants.

In this study, an alternative extraction technology, the Naviglio Extractor®, was employed with the intent to obtain natural antioxidants from wine-waste using a green chemistry approach. The extracts from Cagnulari grape marc obtained with the Naviglio was chemically characterized for the polyphenols content by means of spectrophotometric analysis, High-Performance Liquid Chromatography and Mass Spectrometry. To investigate potential biological activities of the obtained extract, its ability to counteract hydrogen peroxide-induced oxidative stress and cell death was assessed in two human endothelial cell models. Cytotoxic test failed to show any deleterious effect of the extract on cultured endothelial cell, while analysis of the extract for its antioxidant activity revealed a strong antioxidant potential on both cellular models, as indicated by the inhibition of hydrogen peroxide-induced ROS generation and oxidative-elicited cell death. To our knowledge this is the first report on the polyphenolic composition and biological activities of the wine-waste from Cagnulari using the Naviglio® extraction method.
Acknowledgments: Study funded by the Sardinia Region through Sardegna Ricerche Technology Park. Partial support also came from Banco di Sardegna Foundation and L.R. 7/2007 call 2008. AMP was supported by Ricerca cofinanziata PROGRAMMA OPERATIVO FSE SARDEGNA 2007–2013-L.R.7/2007.

P18-32
Influence of diet with cadmium content on the activity of selected enzymes and metallothioneins's level in blood serum and liver of laboratory rat

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Cadmium, one of the most toxic heavy metals, is widely used in many industries. Cadmium enters to the human body particularly by ingestion of contaminated food or by passive inhalation or by tap water. In the organism causes formation of reactive oxygen species (ROS). Rats represent the model organism for assesing the bioavailability of cadmium from contaminated nutriend. We studied influence of diet containing cadmium on the activities of selected antioxidative enzymes in blood plasma of laboratory rat methallothioneins (MT) in serum and liver parenchyma. Cadmium was administerd to experimental animals in the form of diet with 10% poluted soil suplementation from selected localizations or in tap water in dose 20 mg CdCL2.1. Soil in diet served as real model of cadmium incidence in given localities. Positive control was prepared by intraperitoneal injection of CdCl2 solution.

Content of heavy metals in liver and kidney of experimental animals was monitored by atomic absorption spectrometry. There was found significant influence of cadmiumcontent in tap water in the group of first male generation of Wistar strain of laboratory rat on the specific thioredoxinreductase activity in blood plasma.Exposition to cadmium was showing signs of influence on specific thioreductase activity in blood plasma in second filial generation which was fed either by content of cadmium of soil from Suchdol district in diet or tap water with content of cadmium chloride. Drinking of tap water containing cadmium chloride led to decrease of specific catalase activity in male and

female of Wistar strain in first generation. This work was supported by the grant project IAA 600110902 and specific university research (MSMT No21/2012).

P18-33
Can monosodium glutamate accelerate degeneration of the islets of Langerhans in rat pancreas?

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Background: An injection of monosodium glutamate (MSG) into newborn mice caused several features of metabolic syndrome such as obesity, dyslipidemia, and hyperglycemia. We aimed to investigate the effect of long term oral MSG intake on rat pancreas.
Methods: Twenty adult male rats were divided into two groups, control and treatment. Both groups were fed *ad libitum* with a standard rat chow pellet and water. However, the drinking water of the treatment group was mixed with MSG to meet the dose of 2 mg/g body wt./day. Animals were sacrificed after 6 months of MSG ingestion. Fasting blood sugar and histopathology of pancreas were examined.
Results: The H&E staining revealed the greater number of abnormal islets of Langerhans in treatment group compared to the controls. These include macrophage infiltration, islets vascular congestion, vacuolation of beta-cells and fibrosis. There is no significant difference in fasting blood sugar between control and treatment groups.
Conclusion: The abnormal changes found in MSG-treated rat resemble the degeneration of pancreatic islets found in rodent models of T2DM. However, further study needs to be performed.
Keywords: Monosodium glutamate, pancreas, rat, T2DM

P19 – Perinatal Biochemistry

P19-1
Neuregulin involvement in insulin sensitivity

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Background: Neuregulin is an EGF-growth factor family member involved in regulation of muscle metabolism in an acute and chronic manner. Neuregulin signals through tyrosine kinase receptors ErbB2 and ErbB4 in muscle. Chronically, neuregulin induced an oxidative metabolism based on increases in the mitochondrial biogenesis, OXPHOS subunits protein levels and improving insulin sensitivity according to studies in L6E9 myocytic cells culture (Cantó et al. Diabetes, 2007).
Objectives: To study the relevance of neuregulin action in the induction and sustainment of an oxidative metabolism and insulin sensitivity in skeletal muscle
Methods: In donated mice Cre-LoxP knockout (KO) colony (Hans R. Brenner, U. of Basilea) for ErbB2 and ErbB4, muscle conditional (HSA-Cre recombinase), we characterized metabolically double ErbB2 and ErbB4 KO mice.
Results: Contrarily to expected, double KO (dKO) for ErbB2 and ErbB4 mice were highly insulin sensitive according to glucose tolerance test and insulin tolerance test. They showed minor depots of adipose tissue, although maintaining the same ingest rate than control, and a higher oxidative capacity for glucose. Acute exercise in treadmill was well-performed by dKO with increases in glycogen mobilization, glucose consumption and lower levels of lactate release than control animals. Since ErbB2 (proto-oncogen *c-neu*) is an *orphan* receptor, not exclusively related to neuregulin action, but acts as a preferent partner to heterodimerize with ligand-bound ErbB3 and ErbB4 neuregulin receptors, we analyzed phenotype of ErbB2 KO. It showed similar phenotype than dKO. Contrarily, ErbB4 KO mice showed insulin resistance comparing to wild-type mice.
Conclusion: Based on muscle ErbB4 KO, we concluded that neuregulin was required to maintain insulin sensitivity in the whole animal. The high insulin sensitivity phenotype of ErbB2 knockout suggests that this receptor could be associated to insulin resistance situations.
Acknowledgements: Grant: SAF2008-01723 and SAF2011-30467 (& FEDER contribution), Ministerio de Ciencia e Innovación, Spanish Government, to A.G.

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P19r-2
Monitoring pregnancy and prenatal disorders through NMR metabolic profiling of maternal blood plasma

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Firstly, this work presents for the first time, to our knowledge, an NMR metabolic profiling study of pregnancy, through maternal blood plasma. Secondly, the application of a similar strategy to the study of selected prenatal disorders (fetal malformations (FM), chromosomal disorders (CD), pre-diagnostic gestational diabetes mellitus (GDM), pre-diagnostic preterm delivery (PTD) and pre-diagnostic premature rupture of membranes (PROM)) is described.

Multivariate analysis of the [1] H NMR spectra of plasma revealed a clear metabolic trajectory from the non-pregnant state throughout pregnancy. The results reveal an expected increase in total lipids from 1st to 3rd trimester, in tandem with new findings in relation to variations in lipids chain length and insaturation degree. Other metabolite changes include decrease in lactate and increase in glucose, suggesting enhanced gluconeogenesis, as well as lower levels of albumin and choline (newly observed). These and other changes form a novel dynamic metabolic picture of pregnancy. Furthermore, the impact of each disorder mentioned above on blood plasma composition (considering 2nd trimester plasma only, at this stage) was measured, with most significant alterations being found for the FM group (enhanced glycolysis and tricarboxylic acid (TCA) cycle among others), followed by CD and pre-GDM, specifically.

Finally, an overall metabolic picture of pregnancy and prenatal disorders was explored, also for the first time to our knowledge, through Statistical Correlation Spectroscopy (STOCSY) analysis of the NMR spectra of 2nd trimester plasma, in tandem with those of maternal urine and amniotic fluid, as reported previously [1] [2].

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P19-3
Gestational diabetes and obesity: role of oxidative stress and inflammation

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Previous studies from our group have shown that Gestational Diabetes Mellitus (GDM) is associated with an increased oxidative stress, determined as susceptibility of LDL to oxidation. Otherwise, oxidative stress plays an important role in the pathogenesis of obesity. Therefore, the coexistence of both conditions, obesity and GDM, may aggravate the oxidative injury produced in lipids and proteins, and exacerbate the inflammatory process observed in these women. To test this hypothesis we compared in pregnancies complicated by GDM, with and without obesity, and normal pregnancies, oxidative stress, and their correlation with some inflammatory parameters.

During a period of three years women attending the Obstetrics Clinics of Hospital Universitario Fundación Alcorcón were asked to participate in the study. From 2000 gestations we obtained samples from 64 pregnancies complicated by GDM. Between them, 30 were obese (BMI = 31.25 ± 0.7 kg/m^2), and 34 were non-obese (BMI = 23.05 ± 0.4 kg/m^2). Besides, 39 pregnant women without complications were randomly selected as a control group (BMI = 22.28 ± 0.4 kg/m^2). In all women, blood was drawn at 15, 24 and 30 gestational weeks. Vitamin E (vitE), malondialdehyde (MDA), advanced oxidation protein products (AOPP), and inflammatory parameters such as C-reactive protein (CRP) were determined.

In the control group the vitE concentration in the 1st, 2nd and 3rd trimester was 3.2 ± 0.3, 3.1 ± 0.2 and 3.5 ± 0.2 μg/mg lipids, compared with 3.9 ± 0.5, 3.8 ± 0.3 and 4.5 ± 0.4 μg/mg lipids in lean GDM and 3.7 ± 0.5, 4.6 ± 0.6 and 4.3 ± 0.3 μg/mg lipids in the obese GDM group. In control pregnant MDA did not change during pregnancy, whereas AOPP were significantly higher in the 3rd trimester of gestation than in the first part of pregnancy. Similar results were obtained in non-obese GDM. However, in the obese GDM both MDA and AOPP were significantly higher than in the other groups. The AOPP levels in the first and second trimester were positive correlated with the CRP ($p < 0.001$ and $p < 0.05$, respectively).

Our results show that, even in the first trimester, prior to the diagnosis of diabetes, obesity before GDM is associated with an exacerbation in both oxidative stress and inflammation, that could contribute to the complications associated with GDM.

P19r-4
Neuronal differentiation promoted by oleic acid is impaired in TgDyrk1A primary neuronal culture

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Down syndrome (DS) is a genetic disease characterized by the presence of an extra copy of chromosome 21, resulting in a trisomy. Histological brain studies of individuals with DS have revealed an aberrant formation of the cerebral cortex. Some authors attribute this effect to the lack of suitable substrates that direct the migration of neurons. Previous works in our laboratory have shown that oleic acid is a neurotrophic factor that induces neuronal differentiation. Dyrk1A (Dual-specificity tyrosine (Y)-regulated kinase) is one of the genes on human chromosome 21 and mouse chromosome 16 that has aroused most interest, owing to its relationship with the brain functions that are altered in DS. Dyrk1A is overexpressed in DS brains and is involved in neurogenesis and learning/memory. Here we show that oleic acid fails to induce neuronal differentiation in primary neuronal culture from transgenic mice overexpressing Dyrk1A (TgDyrk1A) because the cell clustering and axonal elongation were sharply reduced. Interestingly, the expression of GAP-43, a marker of axonal growth, was increased in the presence of oleic acid in normal neurons but not in TgDyrk1A neurons. To study the mechanism by which oleic acid do not promote cell differentiation in DS models, we used a cell line (CTb) derived from the cortex of trisomic (Ts16) mice where neither oleic acid induces cell differentiation. Dyrk1A was downregulated in CTb by siRNA. In these conditions, oleic acid gave rise to cell clustering and rescued ChAT expression, a marker of cholinergic differentiation, up to similar levels to that of normal cells. Therefore, we can suggest that oleic acid effect is mediated by overexpression of Dyrk1A.

P19-5
Mitochondrial metabolism and hormone action is regulated by proteins participating in mitochondrial dynamics

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Mitochondria are dynamic subcellular organelles that convert nutrient intermediates into readily available energy equivalents. Optimal mitochondrial function is ensured by a highly evolved quality control system, coordinated by protein machinery that regulates a process of continual fusion and fission. Recent work has revealed that proteins participating in mitochondrial dynamics regulate metabolism. Thus, the mitochondrial fusion protein Mfn2 plays an essential role in metabolic homeostasis. Liver-specific ablation of Mfn2 in mice leads to glucose intolerance, and enhanced hepatic gluconeogenesis. Similarly, muscle-specific Mfn2 ablation caused susceptibility to glucose intolerance in response to a high fat diet. Mfn2 deficiency also impaired insulin signaling in liver and muscle due to enhanced ROS production and to ER stress.

The ATP-independent metalloprotease OMA1 plays an essential role in the proteolytic inactivation of the mitochondrial fusion protein OPA1. OMA1 deficiency also causes a profound perturbation of metabolic homeostasis. Thus, ablation of OMA1 in mice results in increased body weight due to increased adipose mass, hepatic steatosis, decreased energy expenditure and impaired thermogenenesis.

Data on the metabolic role of the mitochondrial fission protein Drp1 will be also discussed. All these results reinforce the importance of mitochondrial quality control for normal metabolic function.

P20 – Protein Structure and Function

P20-1
Charged single alpha-helices: identification, role and evolution

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The recently recognized charged single α-helix (CSAH) structural motif (Süveges et al. Proteins 74: 905-916) is characterized by high density of charged residues with a regularly alternating charge pattern. To assess the abundance and significance of this motif, we set up a consensus prediction method that is also available as a web service (at http://csahserver.chem.elte.hu, Gáspári et al. Charged single alpha-helices in proteomes revealed by a consensus prediction approach. Biochem. Biophys. Acta – Proteins and Proteomics 1824: 637–646). A comprehensive search on the UniProt database revealed that CSAHs are quite rare are preferentially associated with proteins involved in symbiosis and RNA binding/processing. In the light of this it is surprising that our analysis of related proteins with CSAH segments showed that the motif is not particularly prone to conservation. We suggest that CSAHs are subject of relatively rapid molecular evolution, also supported by their frequent prediction as disordered and/or coiled coil segments and the properties of regions predicted both disordered and coiled coil forming (Szappanos et al. FEBS Lett. 584: 1623–1627). We propose that rapidly evolving CSAH segments can contribute to the emergence of novel functions.

P20-2
Glutaminase enzyme production from *Hypocrea jecorina*

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In recent years, glutaminase enzyme has attracted much attention in both pharmaceutical and food industrial applications. In food industry, glutaminase is used as a flavor enhancer by increasing glutamic acid content in food through hydrolysis of glutamine to glutamic acid and ammonia. It also used in enzyme therapy for cancer especially for acute lymphocytic leukemia. Another important application of L-glutaminase is in biosensors for monitoring the glutamine levels in mammalian and hybridoma cells (Balagurunathan et al., 2010). *Hypocrea jecorina* (anamorph *Trichoderma reesei*) is one of the most studied and industrially important cellulolytic fungi. In this study, glutaminase enzyme was produced from *Hypocrea jecorina*. The effect of various factors such as pH, temperature, heat and storage stability on the glutaminase enzyme from *Hypocrea jecorina* were investigated. Optimum pH values were 8.0 for glutaminase. The optimum temperature for glutaminase from *Hypocrea jecorina* was found 50°C on standard analysis conditions. Heat inactivation studies showed a decrease in enzymatic activity at temperatures above 50°C. Thermal inactivation data indicated that apparent activation energies with glutamine substrate were calculated for the enzyme. Km and Vmax values were also determined for *Hypocrea jecorina* glutaminase.

Keywords: Glutaminase, glutamin, *Hypocrea jecorina*, enzyme production, microorganisms

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P20-3
The contrasting effect of macromolecular crowding on protein misfolding

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Amyloid fibrils associated with neurodegenerative diseases can be considered biologically relevant failures of posttranslational quality control maintained by molecular chaperones and proteases. However, the role of crowded intracellular environments on protein misfolding is not clearly understood. We report here that: (i) human Tau fragments, when phosphorylated by glycogen synthase kinase-3β, do not form fibrous aggregates in the absence of a crowding agent but do form fibrils in the presence of a crowding agent; (ii) on the other hand, rabbit prion protein and hen egg white lysozyme do not form amyloid fibrils in the presence of a strong crowding agent but do form fibrils in the absence of a crowding agent. Our data suggest that macromolecular crowding could play an important role in the mechanism of posttranslational quality control. It is known that *in vivo* human Tau protein and human prion protein have the tendency to form fibril deposits in a variety of tissues and they are associated with amyloid diseases, while rabbit prion protein and hen egg white lysozyme do not readily form fibrils and do not cause amyloid diseases. We suggest that proteins that are prone to forming fibrils and are associated with diseases are more likely to misfold under crowded conditions than in dilute solutions. In contrast, proteins that are not disease-associated are less likely to form fibrils in crowding conditions compared to dilute solutions. A possible explanation for the contrasting effect of macromolecular crowding on these two sets of proteins has been proposed.

P20-4
Creating a xylanase a fusion construct capable of polymerization

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The aim of our work is to furnish enzymes with polymerization ability by creating fusion constructs with the polymerizable protein, flagellin, which is the main component of flagellar filaments. The D3 domain of *Salmonella* flagellin, exposed on the surface of flagellar filaments, is formed by the hypervariable central portion of the polypeptide chain. D3 is not essential for filament formation. The concept in this project is to replace the D3 domain with suitable monomeric enzymes without adversely affecting polymerization ability, and to assemble these chimeric flagellins (flagzymes) into tubular nanostructures. To test the feasibility of this approach, xylanase A (XynA) from *Bacillus subtilis* was chosen as a model enzyme for insertion into the central part of flagellin.

With the help of genetic engineering, a fusion construct was created in which the D3 domain was replaced by XynA. The construct was transformated into an IPTG-inducible *Escherichia coli* overexpression strain. The N-terminally His_6-tagged flagellin-XynA flagzyme, purified by nickel-affinity chromatography, exhibited catalytic activity as well as polymerization ability. To improve polymerization properties, a removable GST-tag was conjugated through a TEV protease site to the fusion protein which allows preparation of the untagged flagellin-XynA flagzyme. Our results demonstrate that polymerization ability can be introduced into various proteins, and building blocks for rationally designed assembly of filamentous nanostructures can be created. (This work was supported by the National Development Agency grants TÁMOP-4.2.2/B-10/1-2010-0025 and CK77819).

P20-5
Structural changes of super-folder GFP in the presence of negativelly charged ions

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Fluorescent proteins (FPs) have advanced our understanding of basic biology. FPs have been adopted for routine monitoring of gene activation as well as the selective labelling and analysis of single proteins, cellular organelles and whole cells; for the investigation of protein-protein interactions and other biologically-relevant events occurring in living cells; to sense many important properties of the cellular environment, including pH, ion flux, redox potential. Photoactivatable FPs have enabled multicolour imaging of fixed and live cells with resolution over the difraction limit. Far-red and near-infrared FPs and FPs with large Stokes shifts have greatly facilitated deep-tissue imaging in living animals. The application of FPs as a fluorescent tag for investigation of numerous cellular processes requires a carefull examination of the influence of surrounding environment on FP structure and function.

We have shown previously that small concentration of chemical denaturant guanidine thiocyanate (GTC) exerts a pronounced effect on spectral features of super-folder GFP with no evident changes of protein's tertiary structure. As molecule of GTC contains negatively charged ion of thiocyanate and positively charged ion of guanidine, observed changes can be defined by impact of one or both of these ions. To identify which of them are the main players we undertook the study of sfGFP dynamics by spectroscopic methods in the presence of other agents such as guanidine hydrochloride, sodium chloride and sodium thiocyanate. Our results revealed that observed changes are mainly induced by the negatively charged ions of thiocyanate that presumably bind near the chromophore and inhibit its anionic form.

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P20-6
Preferential binding and orientation of retinitis pigmentosa 2 (RP2) in the presence of phospholipid monolayers

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A severe form of retinitis pigmentosa is linked to mutations of the 350 residues protein RP2 (retinitis pigmentosa 2). The high resolution structure of RP2 was shown to include a β-helix and a α-β domain. The parameters responsible for the modulation of its membrane binding are still largely unknown. The objectives of this research work were thus to characterize the extent of RP2 binding in the presence of different phospholipid monolayers. RP2 and a truncated RP2 (α-β domain alone) were expressed and purified. Then, RP2 binding was monitored by surface pressure measurements which demonstrated that its adsorption kinetics is strongly dependent on the type of phospholipid fatty acyl chain and headgroup. For example, on the basis of its binding parameters, RP2 shows a preferential binding onto saturated phospholipid monolayers, which is consistent with its postulated localization to microdomains. The importance of its β-helix in this binding was highlighted by the study of the binding parameters of the truncated RP2. Moreover, measurements by infrared spectroscopy suggest that the orientation of RP2 in monolayers is dependent of lipid composition. Finally, Langmuir monolayers were used to characterize the influence of the physical state of phospholipid monolayers on the binding of RP2. The binding parameters of RP2 were shown to be very different when phospholipids are in the liquid-expanded (LE), liquid-condensed (LC) or solid-condensed (SC) states, which is also consistent with its preferential location to microdomains.

P20r-7
Atypical intracellular highly-specific metallocarboxypeptidases (CCPs) responsible for tubulin processing

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Cytosolic carboxypeptidases (CCPs) integrate a recently described metallocarboxypeptidases subfamily with diverse intracellular distribution. The most studied member of this subfamily, CCP1 or Nna1 (Nervous system nuclear protein induced by axotomy), is over-expressed during neuronal damage situations. Moreover, in recessive ccp1 mutant mice, known as pcd (Purkinje cell degeneration) mouse, Purkinje cells, retina photoreceptors and mitral neurons from the olfactory bulb become degenerated because the lack of the enzyme[1]. Therefore, CCP1 arises as a potential biomedical target regarding neurodegenerative and neuroregenerative processes. But CCP1 is not the only promising protein: five additional genes codifying proteins similar to CCP1 were identified in human and mouse genomes, from CCP1 to CCP6[2].

The biological activity of the six subfamily members is studied in our group applying protein engineering, interactomic approaches, structural and cell biology, as well as phylogenetic studies. Microtubules appeared as the most probable substrate, as it has been demonstrated recently[3]. Even thought other regulation-related interactors have been identified in our group, all CCPs would have a remarkable role on tubulin post-translational modifications (PTMs) at the exposed C-terminal tails of α- and/or β-tubulins. PTMs on tubulin modify microtubules affinity for microtubule associate proteins (MAPs) altering main processes involved in cell survival, cell cycle, as well as neuronal signaling, among others.

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P20-8
Heterologous expression, partial characterisation and theoretical tridimensional structure analysis of the octopine dehydrogenase from the mussel *Mytilus galloprovincialis*

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The opine dehydrogenases (OpDH) are pyruvate oxidoreductases that catalyze the NADH-dependent reductive condensation of pyruvate and an amino acid to produce secondary amine derivatives called opines. In invertebrates, the OpDH protein family includes five enzymes distinguishable from each other by their substrate specificities and which are reported to regulate the cytoplasmic redox balance during exercise and hypoxia. The complete coding region of the putative octopine dehydrogenase (OcDH) from the mussel *Mytilus galloprovincialis* was expressed in the bacteria *Escherichia coli* and the recombinant protein was purified. Enzymatic assays were made to determine the *M. galloprovincialis* OcDH affinities for different amino acid substrates. The protein showed the highest affinity for the amino acid L-arginine (88.22%), compared to L-alanine (9.04%) and glycine (2.74%), while no activity was detected when taurine or b-alanine were used. These data strongly support that this recombinant enzyme is octopine dehydrogenase and not another protein from the OpDH family. The deduced amino acid sequence from de *M. galloprovincialis* OcDH allowed the modelling of a three-dimensional protein structure based on a previously published crystal structure from its homologous counterpart from the great scallop *Pecten maximus*. The superimposition of both models showed interesting changes in the amino acid binding site, which could explain the differences found in the substrate affinity between the enzymes of the two molluscs. These findings are of comparative value since they describe a novel mollusc OcDH, identified in the Mytilidae family for the first time.

P20-9
Features of guanidine hydrochloride interaction with proteins at denaturant low concentrations

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Internationally accepted approach for research processes of protein folding *in vitro* is study their denaturation/renaturation curves under action one or another chemical denaturant. The most frequently for these purposes as a denaturant guanidine hydrochloride and urea are used. Invetigations carried out by us suggest that at protein denaturation by guanidine hydrochloride necessary to consider some features of the interaction of the denaturant with the protein molecules. So, it was shown that low concentrations of GdnHCl may have a stabilizing effect on protein structure. This is caused by the removal of protein spatial structure exertions by guanidine hydrochloride because of electrostatic interactions between the molecules of GdnHCl and the protein. GdnHCl can also aggregation action on different proteins. This effect is based on the interactions of cations GdnHCl ($GuH+$) with $C=O$ groups of glutamic and aspartic acids and in places of localization of amide group of glutamine and asparagine appear positive charges $GuH+$. It creates the conditions for the aggregation of protein molecule to each other. If an excess of positive charges on the surface of the protein molecule on the negative are obtained aggregates dissociate. Apparently, an important contribution to the aggregation of protein molecules in solutions with low concentrations of GdnHCl make hydrophobic interactions. Probably coalescence of hydrophobic clusters in the absence of charge interactions contribute to protein aggregation.

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P20-10
The effect of solvent on protein-ligand interactions

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A tremendous diversity of ligand binding proteins creates considerable opportunities for their scientific and medicinal applications. Ligand-binding proteins of the bacterial periplasm (PBPs) share a characteristic two-lobed structure and undergo a large conformational change as a result of ligand binding. Utilization of the *E. coli* D-galactose/D-glucose-binding protein (GGBP) as the sensitive element in the glucose biosensor is one of the promising directions for continuous glucose monitoring. As GGBP binds glucose with high affinity (K_d = 1 μM), the necessity to lower the affinity of GGBP to glucose should be taken into account in the development of methods to monitor the glucose level in human blood. Still, wild-type GGBP can be used as a sensitive element of biosensor systems in which blood or interstitial liquid sampling is connected with considerable dilution. In this work we considered that interaction of GGBP with its ligand is affected by viscosity of solution. At unfolding-refolding of complex GGBP with glucose (GGBP/Glc) induced by guanidine hydrochloride (GdnHCl) a marked deceleration of the equilibrium acquisition between the native GGBP/Glc and the unfolded protein is seen in contrast to GGBP alone. The presence of glycerin similarly influences on the protein-ligand interaction. This effect is not revealed at heat-induced GGBP/Glc denaturation. These testify that the limiting step of the unfolding-refolding process of the complex GGBP/Glc is the disruption/tuning of the configuration fit between the native state of GGBP and glucose with the process rate depending on denaturant concentration. The obtained data should be taken into account at GGBP using as sensing probe of glucose biosensor.Financial support by Ministry of Education and Science (contract N 16.512.11.2114).

P20r-11
Analysis of electrostatic interactions involving ribosomal and membrane recognition by ribotoxins

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Ribotoxins are a family of toxic extracellular fungal RNases that exert ribonucleolytic activity on the larger molecule of eukaryotic rRNA. They enter the cells and cleave a single phosphodiester bond located within a universally conserved sequence known as the sarcin–ricin loop (SRL). This cleavage leads to inhibition of protein biosynthesis, followed by cellular death by apoptosis. Their ability to interact with acid phospholipids has been related to their transport across the lipid bilayer. Close inspection of the three-dimensional structure of the best known of these

ribotoxins, α-sarcin, reveals the presence of three lysine-rich regions. One of them is loop 3, containing a conserved lysine-rich stretch (Lys111-Lys112-Pro113-Lys114). The elucidation of the structure of three restrictocin co-crystals with substrates mimicking the SRL showed that this sequence contacts a unique prominent G, one of the main determinants of ribotoxins' specificity, which is interpreted as this interaction being required for ribosome recognition. We have therefore substituted these Lys residues by Glu not only confirming these results but also showing their involvement in ribotoxins' membrane interaction. Another lysine rich region would be loop 2. The involvement of this loop 2 in the interaction with lipid vesicles has been also proposed many years ago but only very recently the experiments needed to verify such a prediction have been started by our group. HtA is the smallest fungal ribotoxin known. Interestingly, HtA loop 2 is not only much shorter than α-sarcin's, but almost completely devoid of basic residues, in agreement with the very different behavior of this smaller ribotoxin when encountering acid phospholipid membranes. Accordingly, substitution of the α-sarcin 79–93 (loop 2) sequence stretch by the only four equivalent residues appearing in HtA (Ala-Asp-Ala-Ile) renders a non-specific RNase with a very different ability to interact with lipids, confirming the participation of loop 2 in both recognition processes: passage across the membranes and SRL specific interaction.

P20-12
The effect of point mutations on the stability of human sperm-specific glyceraldehyde-3-phosphate dehydrogenase

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Human glyceraldehyde-3-phosphate dehydrogenase catalyzes one of the glycolytic reactions and exists in two homologous isoenzyme forms: GAPD, a well-studied protein found in all somatic cells, and GAPDS, which is expressed solely in testes. GAPDS supplies energy required for the movement of spermatozoa and is tightly bound to the sperm tail cytoskeleton. Actually, there is only little data on GAPDS properties. GAPDS stability towards denaturation was found to be enhanced as compared to GAPD that may be an adaptation to the absence of protein expression in spermatozoa. In the present study we investigated the structural features of GAPDS accountable for its enhanced stability. At the first step, the GAPD and GAPDS sequences and x-ray structures were computationally analyzed. The following specific features that may affect stability were revealed as a result: (i) seven additional proline residues in GAPDS, with six of them meeting the 'proline rule'; (ii) eight additional glycine residues in GAPD; and (iii) two additional semi-buried interdomain salt bridges in GAPDS. At the second step, the molecular modeling of a series of GAPD and GAPDS mutant variants was carried out and it was shown that all of the features listed above except one of the salt bridges could be somewhat accountable for the GAPDS enhanced stability. At the third step, we used the site-directed mutagenesis approach to introduce mutations to the recombinant GAPDS protein which were assumed to lessen its stability. The subsequent experimental assessment of the stability of the obtained mutant proteins proved our assumptions. The work was supported by the Russian Foundation for Basic Researches (grants 12-04-91330-NNIO_a and 11-08-00663-a).

P20r-13
Immunotoxins aimed against colon cancer: characterization and optimization

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Colon cancer is one of the most deadly types of cancer with a significance incidence in worldwide population. Many different immunotherapeutic approaches have been lately developed against tumoral processes. Among them, immunotoxins based on toxic proteins linked to an antibody moiety hold promise as antitumoral agents, due to their high specificity against targeted cancer cells. We have design three immunotoxins based on the variable fragments (scFv) of the humanized mAbA33, which recognize the glycoprotein GPA33 a well-known colon cancer marker, fused to the fungal ribotoxins α-sarcin or hirsutellin, and the non-toxic RNase T1. The immunotoxins were produced in the yeast *P. pastoris*, purified with high yield, and proven to be properly folded, active, specific and stable. The three immunotoxins showed high specific toxicity against the targeted cancer cells, with small differences in their citotoxic efficacy. Here we discussed the results obtained related to functional characterization, which involves entry into the cell, intracellular trafficking, toxic domain release and target cell death.

P20m-14
Structural studies on the recognition of proline-rich ligands by SH3 domains

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Transient protein–protein interactions are important in controlling cellular networks and have been extensively studied. However, the role of water molecules in these inter-protein interactions is poorly understood. The recognition of proline-rich sequences by protein-protein interaction modules is one of the most common mechanisms. From these modules, the SH3 domain (Src homology domain 3) is one of the best characterized and is present in a large number and variety of proteins participating in cellular signal transduction. Inhibitors of the interactions between SH3 domains and their partners have proved to be promising therapeutic agents, validating these domains as attractive targets for drug design. Nevertheless, in spite of the wealth of structural and functional information collected during the last two decades, the forces driving proline-rich ligand recognition by SH3 domains are still not fully understood. In the last years, our group has developed a team effort to unveil the molecular basis of these interactions through a combined thermodynamic-structural approach. Thermodynamic studies on different SH3 domains have shown a high negative binding enthalpy, which might be explained by the presence of several fully buried water molecules at the binding interface.

In order to analyze the presence and involvement of water molecules in the binding interface of the complexes between the SH3 domain and several synthetic high affinity proline-rich peptides, we have solved the structures of several wild-type and mutated SH3 domains and their complexes. In this work, we analyze how crystallographic parameters can affect to the water molecules modeled in these structures.

P20r-15
Random mutagenesis analysis of the actinoporin StnI from *Stichodactyla helianthus* reveals the importance of particular protein regions for its hemolytic activity

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Actinoporins constitute a family of toxic proteins stored within the nematocysts of sea anemones. They are produced as single polypeptide chains of around 175 amino acids and basic isoelectric point values. In water solution, actinoporins remain mostly monomeric and stably folded but, upon interaction with lipid membranes of specific composition, become oligomeric integral membrane structures. They demonstrate how an identical amino acid sequence can fold into two different structures, showing the environmental influence on the energy landscape of a protein. Actinoporins belong to the much larger group of widely distributed pore forming toxins (PFTs), proteins whose toxic activity relies on the formation of pores within biological membranes. They interact with membranes containing sphingomyelin (SM), displaying phase coexistence, or both, in the absence of any known protein receptor and show hemolytic activity. The sea anemone *S. helianthus* produces at least two different actinoporins (StnI and StnII) with very similar structure but quite distinct hemolytic potency. Using a rapid screening method to analyze randomly produced mutant variants of StnI we have selected, produced, isolated and characterized several mutant variants of diminished activity. The results obtained are discussed in terms of structure-function relationships taking into account the recently determined three-dimensional structure of StnI in solution. The mutated residues appear at strategic locations from structural and functional points of view, shedding light on the mechanism of action of these interesting toxic proteins.

P20-16
Recombinant cytoplasmic domain of the human neuregulin 3 expressed in *Escherichia coli*

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The neuregulins (NRGs) are cell-cell signaling factors with a wide range of function in the nervous system. They belong to the Epidermal Growth Factor superfamily and are ligands for receptor tyrosine kinases of the ErbB family. At present, four different NRG genes (*NRG1-4*) have been identified in vertebrates. Recent studies have demonstrated that polymorphic variation in gene encoding NRG3 affect its expression in human brain and are associated with some neurodevelopmental disorders. Although NRG3 appears to be involved in these diseases pathogenesis, biological role and structure of this protein are poorly characterized. Our experiments were designed to facilitate exploration of the interrelationship between function and molecular properties of cytoplasmic domain of human NRG3 isoform b (NRG3b-ICD). In these studies we show results of bioinformatic analysis of the primary structure of NRG3b-ICD, which suggest the presence of at least three intrinsically disordered regions. These regions could allow NRG3b-ICD to interact with many different proteins present in the cytoplasm and lead to transduction of a so-called reverse signal into the NRG3b-expressing cell. However, all these speculations need to be confirmed experimentally using recombinant homogenous proteins. Toward this two constructs containing cDNA encoding NRG3b-ICD with His- or MBP-His-Tag were obtained and their expression was analyzed in *Escherichia coli*. Low incubation temperature (29°C) of the culture allowed to raise the yield of the protein expression, raised its solubility and reduced the degradation. This enables further elaboration of purification procedure and in consequence determination of molecular determinants of NRG3b-ICD function including bidirectional signaling.

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P20-17
Deciphering of the molecular programming of polyketide synthases (PKS) of pathogenic mycobacteria – determinants of the PKS substrate specificity

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Introduction: The polyketide synthases (PKS) in pathogenic bacteria (i.e. mycobacteria) are responsible for the production of secondary metabolites, the polyketides, which increase the adaptation of microorganisms to well defined ecological niches. The PKS are essential for viability and virulence of certain pathogens, thus they are considered as potential therapeutic targets. There are two levels of PKS molecular programming. First one, determining the general character of produced metabolite, defined by the repertoire of catalytic domains, is well studied. Second one, determining the starter/extender units, stereochemistry and reaction cycle, depends on the specific of catalytic domains, but details of this determination remain unknown.
Study objectives: The aim of the study was to identify specificity determinants of polyketide synthases (Mas and PpsC) from *Mycobacterium tuberculosis*.
Methods: Protein primary structures were aligned using BlastP. Point mutations were introduced into proteins by site-directed mutagenesis. Subsequently His-tagged proteins were produced and purified to homogeneity by IMAC. High resolution protein structures were solved using X-ray crystallography. Enzyme specificity was tested by SDS-PAGE using (^{14}C) radiolabelled substrates.
Results: Alignment of PKS/FAS amino acid sequences and mapping of the differential residues on solved high resolution structure of model *Escherichia coli* FabD, led us to identification of sites of the acyltransferase domain of Mas (AT-Mas) and PpsC (AT-PpsC), putatively associated with the specificity for MMal-CoA and Mal-CoA, respectively. Generated mutations changed biophysical characteristics of AT domain variants and led to modulation of the enzyme specificity, either broadening (AT-Mas S726F, N776; AT-PpsC P761S) or inversing it (AT-Mas M624V S726F). Crystal structures of both domains were solved at 3.0 Å and 1.8 Å resolution, and obtained data supported experimental results.
Conclusions: The determinants of the PKS specificity were identified.

P20r-18
Structural screening of cupredoxins against malaria

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The increasing resistance of malarial parasite to current drugs makes the discovery of novel antimalarial agents and the development of effective vaccines urgent needs. Thus this work is aimed at investigating the ability of cupredoxins (azurin, plastocyanin and rusticyanin) to inhibit the spread of parasitemia in red blood cells infected by *Plasmodium*. The rationale for this screening is the structural homology of such cupredoxins with the F_{ab} fragment of the antibody against $MSP1_{19}$, which is responsible for heme degradation inside food vacuoles and the major protein component on the surface of blood-stage parasite[1]. Previous studies have also demonstrated (i) the ability of cupredoxins to enter mammalian cells[2] and (ii) the antimalarial activity[3] of azurin upon binding to $MSP1_{19}$.

Our NMR titrations, ITC experiments and BIGGER-based docking calculations reveal that the acidophilic bacterial rusticyanin, unlike other cupredoxins, specifically interacts with $MSP1_{19}$. It is the holo-rusticyanin rather than the apo-protein which binds to $MSP1_{19}$. The latter in turn uses the same surface area to bind the copper protein as to block the antimalarial antibody[4].

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P20r-19
Construction of a mimetic mutant of cytochrome *c* phosphorylated at tyrosine 48 to further study its biological role in the transition from cell life to death

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Post-translational modifications of proteins are relevant regulatory mechanisms to control an ample number of processes involved in cell metabolism. One of the most usual modifications is phosphorylation, which alters the electrostatic and structural features of proteins in such a way that it affects their interactions with other proteins.

In the particular case of cytochrome *c* (C*c*), its double role – in transferring electrons from the cytochrome bc_1 complex to cytochrome *c* oxidase into the respiratory electron transport chain and in triggering programmed cell death (PCD) under oxidative stress – is post-translationally regulated by nitration or phosphorylation at tyrosine 48.[1–3]

Since the specific C*c*-phosphorylating kinase is still unknown, we have constructed a phosphomimetic mutant of C*c* in which the Tyr48-encoding triplet is substituted by an amber codon that allows the incorporation into the protein of a non-canonical amino acid (*p*-carboxymethyl-L-phenylalanine, *p*CMF). Such a mutation has drastic consequences not only on the C*c* structure (3D conformation, thermal stability, redox potential), but also on its biological function (O_2 consumption, binding to cardiolipin).

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P20-20
Molecular mechanism of the fork reversal by Rad5

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Replication forks may stall as a result of unrepaired DNA lesions or premature dissociation of the components of the replication machinery. Stalled forks are unstable and their collapse is extremely dangerous, as it may result in double stranded DNA breaks.

Rad5 is a yeast protein with human homologs having a proposed replication fork reversal activity, which may facilitate the restart of stalled replication forks. Rad5 is a SWI/SNF ATPase having no canonical helicase activity. It does not unwind unbranched dsDNA substrates, but it can process four-armed forks with homologous arms.

Our study is aimed at deciphering Rad5 mechanochemical activities. Beside full-length Rad5, we also used an N-terminal truncated construct lacking 160 aminoacids. The relatively low basal (DNA-free) ATPase activity of Rad5 is accelerated by both ssDNA and dsDNA. Poly-dT ssDNA and dsDNA enhance this activity 5 and 50 times, respectively. This difference suggests that the enzyme acts differently on the two types of substrate. We observed limited ssDNA length dependence of the ATPase activity. Our results indicate a large Rad5 effective binding site of 60 nt.

Some of our measurements indicates that the Rad5 is not a processive motor. Now we are investigating the activity of Rad5 on a new substrate, designed for short-distance fork reversal assays. We are employing the advantage of single round conditions to shed light on the molecular mechanism.

P20-21
Multimerization domains of insulator proteins of Drosophila melanogaster in long-range genomic interactions

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BTB domain of Drosophila GAGA-factor (GAF) is a rare example of isolated protein domain generally thought able to support

long-distance interactions (over 1kb) in the genome, and the only BTB domain found to form multimers, whereas others are dimers. We demonstrated that a group of BTB domains including Mod(mdg4), Tramtrack, Pipsqueak and Batman, are also able to form multimers (oligomers) like GAF. Cross-linking experiments revealed presence of major multimer corresponding to the 8mer much likely composed with canonical BTB-dimers. Conserved in this group N-terminal hydrophobic sequence is essential for multimerization. Surprisingly we didn't reveal ability of GAF to support long-distance (at 5 kb) genomic interactions in well-established fly model system in contrast to conserved insulator protein CTCF and other Drosophila insulator proteins Zw5 and Su(Hw). Limited proteolysis followed by MALDI-TOF MS analysis and cross-linking experiments revealed presence of dimerization domain in the N-terminal part of Drosophila CTCF. Zw5 protein also have conserved dimerization module. In contrast human CTCF doesn't possess such domain, neither do Su(Hw). Universal component of insulator protein complexes – CP190 contains dimerizing BTB-domain essential for interactions with Su(Hw) and CTCF. Properties of CTCF-CP190 protein complex were studied, its stoichiometry based on cross-linking data revealed inability of dimerizing CP190 BTB to linking insulator proteins molecules together. Considering these facts we conclude that dimerization is sufficient to specifically support long-range interactions in combination with unknown factors.

P20-22
Biochemical characterization of autophagin-2 from the parasite *Trypanosoma cruzi*

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Eukaryotes from yeast to human recycle their intracellular components through the vacuoles/lysosomes in an evolutionary conserved pathway termed autophagy. Autophagy is a process, in which the cellular components are enwrapped by the autophagosome, and are delivered to the vacuole/lysosome for breakdown. The core machinery of autophagy was found to be strikingly similar in mammals and unicellular organisms, such as protozoans. The parasite *Trypanosoma cruzi* causes Chagas disease, a prevalent health problem widespread in Latin America. Its life cycle alternates between an insect vector and a mammalian host and involves four developmental stages. During the differentiation massive morphological changes occur, where autophagy plays a crucial role in the metabolic digestion of excessive and waste cellular components. One of the key steps in autophagy is the proteolytic removal of the C-terminus from the Atg8 protein by the cysteine proteinase Atg4 (autophagin), thereby exposing a glycine residue. The processed Atg8 is inserted into the phagophore membrane, where it enables autophagy progression. Our aim was to map the active-site cleft and to identify optimal synthetic substrates for the autophagins. We took advantage of combinatorial substrate libraries in order to identify synthetic substrates that will enable the biochemical characterization of the autophagins. Based on the experimental screens of the tetrapeptidic libraries an optimal substrate was synthesized, which was found to be cleaved exclusively by the *T. cruzi* autophagin-2 and not by the human autophagin-1. Therefore, this provides an opportunity for a high-throughput screening of compounds targeting *T. cruzi* autophagins with the aim of preventing the pathogenesis of Chagas disease.

P20-23
Synthesis of *E. coli* microcin C and its homologs encoded by other bacteria in an *in vitro* system

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Microcin C (McC) is a potent antibacterial agent produced by some strains of *Escherichia coli* McC consists of a ribosomally synthesized heptapeptide with a modified AMP attached through a phosphoramidate linkage to the αserif-carboxyl group of the terminal aspartate. McC is a Trojan horse inhibitor: it is actively taken inside sensitive cells through YejABEF transporter. Once inside, McC is processed and the product of processing, a nonhydrolyzable aspartyl-adenylate, inhibits translation by preventing aminoacylation of tRNA ^{Asp} by aspartyl-tRNA synthetase (AspRS).

There are six genes in McC gene cluster (*mccABCDEF*). The first gene, *mccA*, encodes the pro-McC heptapeptide. The second gene, *mccB*, encodes an enzyme that catalyses an attachment of AMP posttranslational modification of the MccA heptapeptide; other genes from the cluster encode self-immunity proteins. Clusters of genes homologous to McC biosynthesis and immunity genes were revealed in various bacteria using bioinformatics tools and genes coding for putative pro-peptides of various length were predicted.

To determine if *E. coli* MccB can attach AMP to variants of MccA heptaptide MRTGNAN of different lengths, extended peptides GMRTGNAN, GGMRTGNAN, GGGMRTGNAN etc. were tested in an *in vitro* McC synthesis system. We found that the addition of up to 10 glycine residues to MccA did not affect the adenylation reaction and resulting peptide adenylates were biologically active. Moreover, MccB was able to adenylate proteins with C-terminal GGGGMRTGNAN tag, opening way for C-terminal labeling of various proteins.

We have cloned and purified MccB homologs encoded by putative McC biosynthetic clusters from *Synechococcus* sp. and *Streptococcus thermophilus* and tested their activity *in vitro* with chemically synthesized putative precursor peptides. In both cases, the products of adenylation reactions were observed, validating bioinformatic predictions. Determination of biological functions of McC-like molecules encoded by *Synechococcus* sp. and *S. thermophilus* is currently in progress.

P20-24
The hexameric structures of human heat shock protein 90

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The human 90-kDa heat shock protein (HSP90) functions as a dimeric molecular chaperone. HSP90 identified on the cell surface has been found to play a crucial role in cancer invasion and metastasis, and has become a validated anti-cancer target for drug development. It has been shown to self-assemble into oligomers upon heat shock or divalent cations treatment, but the functional role of the oligomeric states in the chaperone cycle is not fully understood. Here we report the crystal structure of a truncated HSP90 that contains the middle segment and the carboxy-terminal domain, termed MC-HSP90. The structure reveals an architecture with triangular bipyramid geometry, in which the building block of the hexameric assembly is a dimer. In solution,

MC-HSP90 exists in three major oligomer states, namely dimer, tetramer and hexamer, which were elucidated by size exclusion chromatography and analytical ultracentrifugation. The newly discovered HSP90 isoform HSP90N that lacks the N-terminal ATPase domain also exhibited similar oligomerization states as did MC-HSP90.While lacking the ATPase domain, both MC-HSP90 and HSP90N can self-assemble into a hexameric structure, spontaneously. The crystal structure of MC-HSP90 reveals that, in addition to the C-terminal dimerization domain, the residue W320 in the M domain plays a critical role in its oligomerization. This study not only demonstrates how the human MC-HSP90 forms a hexamer, but also justifies the similar formation of HSP90N by using 3D modeling analysis.

P20-25
Laboratory evolution of superior inteins for protein semisynthesis

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Chemoenzymatic approaches bear potential advantages over chemical ligation methods in the generation of site-selectively modified proteins. Two polypeptide segments, of which one is synthetically and the other recombinantly produced, can be linked through protein *trans*-splicing to give a semisynthetic protein.

Inteins remove themselves out of precursor proteins and its activity is being exploited for many unique tools useful for the manipulation of proteins. Correspondingly, inteins have become increasingly important in recent years in many fields of biochemistry, chemical biology, biotechnology, and biomedicine, and are used by a large and growing number of laboratories. However, a major drawback of inteins is their dependence on the native amino acid sequence. In particular, the few amino acids directly flanking the intein when inserted into or fused to a heterologous protein can have a large impact on the inteins' activity. Thus, amino acid substitutions in the protein of interest have to be made that may severely restrict the potential of the intein technology.

To overcome these limitations we apply directed protein evolution methods to inteins. Using a sequential directed evolution method one of the selected mutants of the *Ssp* DnaB intein showed an about 60-fold increased first-order rate of *trans*-splicing compared to the parent intein.[1] The intein mutants we describe largely overcome the above mentioned limitations and show a path to the development of 'super-inteins' that may be active in any given sequence context.

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P20-26
The interaction of the hydrophobic dye ANS with inactivated actin

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Actin is the most abundant protein in eukaryotic cells. Previously, we have shown that the so-called inactivated actin, which for a long time was considered to be an intermediate state in the pathway of protein unfolding, actually is a monodisperse ordered aggregate, binding ANS. This study aimed to determine the parameters of ANS binding to inactivated actin. It was shown that, despite of a significant increase in fluorescence intensity and blue shift of the fluorescence spectrum (from 530 to 470 nm) of ANS solution in the presence of inactivated actin, its absorption spectrum did not change, though fluorescence excitation spectrum was red shifted (λmax = 385 nm) as compared with that of free dye (λmax = 340 nm). It was shown that the position of fluorescence excitation spectrum and fluorescence spectrum of ANS solution in the presence of inactivated actin changed even at low concentrations of protein and remained unchanged with further increase in protein concentration, while the fluorescence intensity increased linearly. These data suggest that in solution of ANS in the presence of inactivated actin, there are always two kinds of dye molecules: free dye and dye interacting with protein, which differ by fluorescence and absorption spectra. The quantity of ANS molecules interacting with protein is so small that it does not have any effect on the absorption spectrum of the solution, but due to the high fluorescence quantum yield of these molecules they determine its fluorescence and fluorescence excitation spectra.

P20-27
Evidence for chemoreceptors with bimodular ligand binding region: signal binding to each module causes a response

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Chemoreceptor based signalling is a central mechanism in bacterial signal transduction. Receptors can be classified according to the size of their ligand binding region (LBR). The well-studied cluster I proteins have a 100–150 residue LBR that contains a single site for the recognition of chemoattractants. Recent genome analyses reveal that cluster II receptors, which contain a 220–300 residue LBR, are almost as abundant as cluster I receptors. However, this family remains largely uncharacterized. We report here high-resolution structures of the LBR of the cluster II McpS chemoreceptor of *Pseudomonas putida* KT2440 in complex with different chemoattractants. This receptor has been shown to mediate chemotaxis towards Krebs cycle intermediates. The structure of McpS-LBR represents a novel small-molecule binding domain. McpS-LBR is composed of two modules, each containing a ligand binding site. Ligand binding was verified by microcalorimetric titrations of site-directed mutants. Malate and succinate were found to bind to the membrane-proximal module whereas acetate binds to the membrane-distal module. In a structural alignment of both modules both ligand binding sites coincide and amino acids in both binding sites are conserved. Chemoattractant binding to both modules was found to trigger chemotactic responses. Both binding sites are connected by a long helix, which is proposed to function as a signaling helix. The bimodular arrangement of the LBR corresponds to a novel type of chemoreceptor which permits responses to two different types of ligand. We propose that the duplication of a structural module of the LBR is an alternative strategy to increase the number of signal molecules recognized by a bacterium.

P20-28
A new trend in the experimental methodology of amyloid fibril structural investigation with the use of thioflavin T

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Amyloid fibrils are insoluble protein aggregates deposition of which accompanies a range of serious diseases, e.g. Alzheimer's and Parkinson's diseases, type II diabetes and Creutzfeldt-Jakob disease. Benzothiazole dye thioflavin T (ThT) is widely used for diagnostics of amyloid fibril formation *in vivo* and *in vitro* due to the unique property of this dye to form highly fluorescent complexes with amyloid and amyloid-like fibrils. It has now become evident that ThT can also be used for structural investigations of amyloid fibrils and even for treatment of amyloid diseases. In this connection, our work is devoted to elaboration an approach for accurate determination of ThT–amyloid fibril binding parameters and characteristics of bound dye: the number of binding modes; stoichiometry and binding constant for each mode; absorption spectrum and molar extinction coefficient for ThT bound to the sites of each mode. The proposed approach was used for examination of ThT interaction with lysozyme, insulin, Aβ42 peptid and β2-microglobulin amyloid fibrils. Obtained characteristics of ThT – amyloid fibril interaction can be used for studying and comparing the structure of amyloid fibrils formed by different amyloidogenic proteins. This work was supported by Program MCB RAS, by grants RFBR-Bel 12-04-90022 and RFBR 12-04-01651.

P20-29
Dimerisation and co-operativity of endoplasmic reticulum aminopeptidase 1 and 2: a process integral to antigen recognition

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Endoplasmic reticulum aminopeptidase 1 and 2 (ERAP1 and ERAP2) are 120 kDa membrane-bound zinc M1 aminopeptidase's that play a central role in the final processing of MHC class I antigens. They are responsible for trimming precursors to antigenic peptides in the endoplasmic reticulum, which is essential to customise the longer peptides to the correct length for presentation on MHC class I molecules. Here we show that recombinantly purified ERAP1 and ERAP2, while monomeric in isolation, readily and specifically form a stable heterodimer through their N-terminal catalytic domains, with the active sites facing each other (face-to-face) and the C-terminal domains acting as a molecular gate above the active sites. While the individual enzyme's substrate specificity is quite limited, the heterodimer shows broad substrate specificity and a cooperatively enhanced level of activity, particularly against native substrates requiring multiple residues to be removed. The heterodimer also produced a greater proportion of fully processed products, and less of the intermediates, although this was dependent on the presence of the C-terminal domains which help retain the intermediate products within the dual active site environment, enabling more complete proteolysis. While the ERAP heterodimer is unique among the M1 aminopeptidases, it is an effective mechanism to efficiently and completely process a broad range of substrates to their appropriate lengths for antigen presentation.

P20-30
A non-damaging method to analyze the configuration and dynamics of nitrotyrosines in proteins

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Often, deregulation of protein activity and turnover by tyrosine nitration drives cells toward pathogenesis. Hence, understanding how the nitration of a protein affects both, its function and stability, is of outstanding interest. Nowadays, most of the *in vitro* analyses of nitrated proteins rely on the chemical treatment of native proteins with an excess of a chemical reagent. One of them – peroxynitrite – stands out for its biological relevance. Still, given the excess of the nitrating reagent, the resulting *in vitro* modification could differ from the physiological nitration. Here, we determine unequivocally the configuration of distinct nitrated tyrosine rings in single-tyrosine mutants of cytochrome *c*. We aimed to check the nitration position by a non destructive method. Thus, we have resorted to ^{1}H-^{15}N HSQC spectra to identify the $^{3}J_{N\text{-}H}$ correlation between a ^{15}N tagged nitro group and the adjacent aromatic proton. Once the chemical shift of this proton was determined, we compared the region ^{1}H-^{13}C HSQC spectra of untreated and nitrated samples. All tyrosines were nitrated at ε positions, in agreement to previous analysis by indirect techniques. Notably, the various nitrotyrosine residues show a different dynamic behavior that is consistent with molecular dynamics computations.

P20-31
Recognition of amino acids by the chemosensory system of *P. aeruginosa*

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Chemotaxis of enterobacteria towards amino acids has been extensively studied and serves as the principal model system in the field. The paralogous receptors PctA, PctB and PctC of the human pathogen *Pseudomonas aeruginosa* have been reported to mediate chemoattraction to amino acids and intermediates of the amino acid metabolism as well as chemorepellation against some chlorinated hydrocarbons. Here we report a study of the purified, recombinant ligand binding regions (LBRs) of these three receptors. Isothermal titration calorimetry (ITC) experiments showed that PctA-LBR and PctB-LBR bind 17 and 5 L-amino acids, respectively. L-Gln was one of the amino acids which did not bind to PctA-LBR but was by far the tightest binding ligand of PctB-LBR, which suggests that PctB has evolved to specifically bind L-Gln. No binding of intermediates of amino acid metabo-

lism and chemorepellents was observed for any of the proteins analyzed. Homology modeling has shown that the three LBRs form a bimodular structure similar to double PDC like domains. The analysis of site-directed mutants of PctA-LBR demonstrated that ligands bind to the membrane distal module. Differential scanning calorimetry studies showed that proteins unfold in a single event and that different ligand binding modes observed by ITC were reflected in different magnitudes of ligand-induced protein stabilization. Analytical ultracentrifugation studies demonstrate that proteins are monomeric in the absence and presence of ligands. It can be concluded that the molecular basis of amino acids chemosensing in enterobacteria and *P. aeruginosa* are entirely different.

P20-32
Phosphorylation by CK2 regulates the binding of human centrins to the cellular targets

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Centrins are calcium-binding proteins involved in several cellular processes and are the targets of several protein kinases (PKA, Aurora, CK2). Phosphorylation by CK2 at T138 has been suggested to regulate the binding of vertebrate centrin 1 to transducin protein in retina (1). Two other cellular targets bind to centrins: Xeroderma Pigmentosum Group C (XPC) protein (involved in recognition of DNA damage) and Sfi1 protein (involved in centrosome duplication) via a helicoidal and hydrophobic motif $W^1L^4L^8$ in the case of XPC, $L^8L^4W^1$ in the case of Sfi1 (2) and $W^8L^4W^1$ in the case of transducin. Our main objective is to analyze the phosphorylation mechanism catalyzed by CK2 on human centrins and to describe its consequences at the structural level as well as the target binding of centrins. One phosphorylation site by CK2 has been identified in the C-terminal domain of centrin 1 and two sites in the C-terminal of centrin 2. Site-directed mutagenesis used either to block or to mimic one or two phosphorylation sites, allowed us to analyze the phosphorylation effect. The binding capacity of centrins to their targets (XPC, Sfi1, transducin) of CK2-phosphorylated centrins 1 and 2 as well as of their variants has been measured by Isothermal Titration Microcalorimetry (ITC).

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P20-33
Covalent attachment of heme to the protein moiety in an insect E75 nitric oxide sensor

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The general architecture of cellular nuclear receptors is well known, and the presence of a ligand binding domain (LBD) can be readily identified when inspecting their primary sequence. Fatty acids, phospholipids, retinoids, thyroid hormones and steroids are among the various compounds that can tightly associate to the LBD of nuclear receptors. It has recently been discovered that the insect nuclear receptor E75 is a heme protein that can bind diatomic gases such as CO or ·NO hence regulating the transcriptional properties of the receptor. We have recombinantly expressed and purified the ligand binding domains of various insect nuclear receptors of the E75 family. Both the *Drosophila melanogaster* and *Bombyx mori* are purified as ferric hemoproteins with Soret maxima centered at 424 nm whereas their ferrous form has a Soret maximum centered at 425 nm that responds to ·NO and CO binding. However, the purified LBD of *Oncopeltus fasciatus* displayed a Soret with a maximum at 415 nm for the ferric protein and 425 nm for its ferrous counterpart. Binding of ·NO to the heme moiety of *D. melanogaster* and *B. mori* E75 LBD results in the appearance of a peak at 385 nm whereas this peak appears at 416 nm in the case of the *O. fasciatus* hemoprotein, resembling the behaviour displayed by their human homolog Rev-erbβ. HPLC analysis revealed that, unlike the *Drosophila* and *Bombyx* counterparts, the heme group of *Oncopeltus* is covalently attached to the protein moiety through the side chains of two amino acids. Sequence homology with *O. fasciatus* lead us to clone and express the LBD of *Blattella germanica* and allowed us to show that it also has the heme covalently bound to the protein moiety. Hence, ·NO regulation of the transcriptional activity of these nuclear receptors might be differently controlled among various insect species.

P20-34
Deciphering the role of Trip3 and the R2TP in the assembly of RNP complexes containing L7Ae protein

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Ribonucleoproteins (RNP) are essential complexes for gene expression and stability of genomes. Small nuclear (sn)RNPs permit mRNAs splicing. Small nucleolar (sno)RNPs and small Cajal body (sca)RNPs direct post-transcriptional modifications of rRNAs and snRNAs respectively. Telomerase is a scaRNP that functions to elongate the telomeres. All these RNAs are assembled with a set of proteins called core proteins, including L7Ae protein. The mechanism of their assembly is not clearly defined.

In the laboratory, we previously described an assembly factor: Nufip, which directly interacts with L7Ae proteins. We recently described another protein: Trip3, also interacting with Nufip. We provide evidences showing that the dimer Nufip/Trip3 is important for the first steps of the assembly of RNPs. By biochemical technics and proteomic approach, we found that Nufip and Trip3 form a complex with core proteins that could later be recruited to nascent small RNAs, and also with proteins from the R2TP complex. Nufip recognizes L7Ae protein on these RNAs and recruit R2TP complex and Hsp90 for correct folding of RNP complex with all core proteins. Previously, a sequential model for the assembly of snoRNP has been described *in vitro*, in which the first step of the assembly occurs between the RNA and the L7Ae protein and then, the other core proteins are assembled into the complex. Our approach highlighted a new model for the assembly of sn(o)RNP complexes in which there is first a protein pre-complex containing several core proteins and the assembly factors Nufip and Trip3 that is RNA free. The entry of RNA would be subsequent to this step. How Nufip, Trip3 and the R2TP could participate in the recruitment of core proteins is currently investigated. Another approach with mutant proteins which block the assembly of the complex at early steps is also used to study the mechanistic of the R2TP complex in this process.

P20-35
Human Papilloma Virus intrinsically disorder protein E7 characterization by NMR spectroscopy

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Many functional proteins are devoid of stable secondary and tertiary structure, and recent evidence shows that these proteins gain functional advantages by remaining natively unstructured. Intrinsically disordered proteins (IDPs) could be either completely or partially unstructured, and were shown to play important biochemical functions including molecular recognition, signaling, and regulation with implication in several human diseases like Alzheimer, Parkinson, cancer and many viral infections. The oncoprotein E7 from HPV-16 has a key role in the development of cervical cancer, the second most common cancer in women worldwide, and it is known to be partially unstructured. It is a 98 residues protein composed by three conserved regions, CR1 and CR2 in the N-terminal half and CR3 in the C-terminal half, and the first 55 amino acids are in a disordered region. This region has been found to interact with a high variety of targets, like the Retinoblastoma tumor suppressor protein, and it is of crucial importance to achieve its structural and dynamical characterization, despite the absence of a stable structure. Nuclear magnetic resonance spectroscopy has been proven to be a useful tool to obtain atomic resolution information on IDPs. In this work we will present the expression, purification and characterization of the full-length HPV-16 E7 protein. The expression was optimized (with yields up to 20 mg/l) to obtain double-labeled (^{15}N, ^{13}C) samples suitable for multinuclear NMR experiments and the results of the experiments carried out in several experimental conditions will be shown. The NMR characterization was complemented by the use of other spectroscopic techniques, providing a framework to understand the functional role of this protein.

P20-36
Biochemical characterization of the hepatitis C virus envelope chimera E2E1Δ268-292

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The hepatitis C virus (HCV) is the main cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Nowadays, an effective vaccine against HCV is not available and current therapies are expensive and partially inefficient. The HCV genome consists of a positive single-stranded RNA of 9500 nucleotides which is translated into a polyprotein encoding structural (C, E1, E2 and p7) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). The E1 and E2 envelope glycoproteins must bear the determinants responsible for the binding to the hepatocytes and for the entry of the virus into the cells, and hence, may constitute potential candidates for a future vaccine against HCV. Using the baculovirus/insect cell system, we have successfully produced a chimeric polypeptide (E2E1), containing the complete ectodomains of both envelope glycoproteins. This chimera is able to bind to human antibodies present in HCV-positive human sera and possesses the ability to destabilize model membrane systems. The objective of this study was to determine the possible role of a described fusogenic peptide in E1 (residues 268-292) by analyzing the properties of the corresponding deletion mutant, E2E1Δ268-292 also expressed in baculovirus system. The studies of isolated E2E1Δ268-292 revealed that the new chimera maintained the secondary and tertiary structures as well as the oligomerization ability of the E2E1 protein. This means that the deletion of 25 amino acids from E1 did not lead to changes in the overall conformation of E2E1. Moreover, E2E1Δ268-292 showed higher antigenicity than E2E1. On the other hand, in lipid interaction studies, the mutant protein induced an increase both in aggregation and in release of aqueous contents of acidic vesicles with respect to the wild type chimera. According to these results, the region 268-292 of E1 seems not to be essential for the fusion between the viral and cellular membranes.

P20-37
Conformational changes of Starmaker protein and its interaction with calcium carbonate crystals

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Otoliths, biominerals existing in fish inner ear are responsible for sensing gravity and perception of sound. Starmaker (Stm) is involved in otoliths formation in zebrafish. It has been shown that Stm is a component of otolith and controls shape, size and polymorph selection of calcium carbonate.

Stm belongs to a group of intrinsically disordered proteins (IDPs). IDPs can fulfill their function in disordered state. IDPs exist as a population of molecules in several different conformations. Moreover, interaction with partner and/or post-translational modifications can trigger conformational changes of IDP to more ordered structure.

Previously, we have shown using *in vitro* biomineralization assay and fluorescence microscopy that Stm is a component of calcium carbonate crystals. However, the distribution of Stm in the crystal was unknown. Two-photon microscopy has shown that Stm exists in whole volume of calcium carbonate crystals, but not equally distributed.

We have shown that Stm binds calcium ions, but phosphorylation by CK2 does not increase significantly this ability. Gel filtration and circular dichroism (CD) spectroscopy has been applied to examine the influence of phosphorylation on the conformation of Stm in the presence and absence of calcium ions. Increasing concentration of the calcium ions causes decrease of the hydrodynamic radius of Stm. The change of radius was slightly higher in case of StmP. CD spectroscopy has shown than the secondary structure of Stm and StmP is very similar. The calcium ions affects slightly the secondary structure, but they do not trigger disorder-to-ordered conformation change.

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P20-38
Small-angle X-ray scattering revealed a structural dynamics of botulinum neurotoxin-associating protein, nontoxic nonhemagglutinin

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Clostridium botulinum produces botulinum neurotoxin (BoNT), which is the most potent poison in nature. In the culture super-

natant, the BoNT exists as a part of the toxin complex (TC) in which nontoxic nonhemagglutinin (NTNHA) and/or hemagglutinins (HAs) are assembled onto the BoNT. A series of the investigations indicated that the formation of the TC is vital role for delivery of the toxin to the nerve cells through the digestive tract. In the assembly process, the BoNT binds to NTNHA yielding M-TC, and then matures into L-TC by further association with the HAs via NTNHA in the M-TC. Thereby the NTNHA molecule plays an important role for the construction of botulinum TC. Here, we revealed a crystal structure of the NTNHA produced by *C. botulinum* serotype D strain 4947 (D-4947) at 3.9 angstroms. Additionally, we performed small-angle X-ray scattering (SAXS) analysis of the M-TC and NTNHA to clarify the solution structure. The crystal structure of the D-4947 NTNHA revealed that the BoNT and NTNHA shares closely related structure consists of three domains. The SAXS image displayed that, even though the probable N-terminal two-thirds of the NTNHA molecule exhibited apparently similar form in both the crystal and solution structures, the probable C-terminal third of the molecule showed extended structure in the SAXS image than that in the crystallographic image. The extended structure was not observed in the M-TC solution structure. The discrepancy between the crystal and solution structures implies the flexibility of the C-terminal third domain of the NTNHA, which is involved in the binding to the BoNT. Structural dynamics of the NTNHA molecule revealed by the SAXS may explain the binding to the BoNT to form the BoNT/NTNHA complex.

P20-39
Analysis of the interaction between desmosomal proteins: Desmoplakin & Plakophilin

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Desmosomes are intercellular junctions that anchor cytoskeletal intermediate filaments at the plasma membrane. This is achieved through a complex network of protein-protein interactions which provide a strong cell-cell adhesion and maintain the structural and functional integrity of tissues [1]. Desmoplakin (DSP) and plakophilins (PKP) are components of these macromolecular complexes. DSP belongs to the plakin family of cytolinkers and contains in its N-terminal region a Plakin Domain –conserved in the whole family– that is formed by eight Spectrin Repeats and an SH3 domain. The Plakin Domain of DSP is important to localize DSP in the desmosomal plaque and to interact with binding partners such as PKPs, which are members of the armadillo protein family [2]. We have designed a collection of DSP plakin domain and PKP1a fragments. The recombinants proteins were co-expressed in COS1 cells and the interactions were studied by co-immunoprecipitation. Our results suggest that the Plakin Domain of DSP harbors more than one interaction site for PKP1.

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P20-40
Calcium and 14-3-3 dependent activation of yeast neutral trehalase NTH1

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Yeast neutral trehalase (NTH1; EC 3.2.1.28) is an enzyme that catalyses the conversion of trehalose (1-α-D-glucopyranosyl α-D-glucopyranoside) into two glucose molecules. This nonreducting disaccharide is considered as an important stress and reserve metabolite. Activity of NTH1 is regulated by several factors: PKA protein phosphorylation, yeast 14-3-3 (BMH1 and BMH2) protein binding and at last by Ca^{2+} binding. Specific EF-like motif D^{114}TDKNYQITIED^{125} is located on the N-terminus of *S. cerevisiae* NTH1. This motif is responsible for calcium binding and is highly conserved in many Ca^{2+}-binding proteins, D114 and D125 are responsible for Ca^{2+}-binding. I121 is important for a correct conformation of the motif. Therefore we prepared four mutants: D125L, K117L, I121L and D125L. For our study we used enzyme-kinetic measurements and isothermal titration calorimetry to reveal how the phosphorylation, the Ca^{2+} and BMH protein binding affect the activity of NTH1 from yeast *S. cerevisiae*.

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P20-41
Structural basis for the 14-3-3 protein-dependent activation of neutral trehalase NTH1

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Neutral trehalase (NTH1) (EC 3.2.1.28) is a yeast enzyme, which hydrolyzes trehalose to two glucose molecules. α,α-trehalose (1-α-D-glucopyranosyl α-D-glucopyranoside) plays an important role as a reserve and stress metabolite in yeast cells, thus NTH1 figures in stress response. The activity of NTH1 is mediated by the yeast isoforms of 14-3-3 proteins – BMH1 and BMH2 and by calcium in smaller extent. When NTH1 is phosphorylated by PKA, it forms a complex with one of the BMH protein in ratio 1:2, which is required for activation of NTH1. However, the exact mechanism of BMH-dependent activation of NTH1 remains still elusive. We used biochemical methods, such as site-directed mutagenesis, enzyme-kinetic measurements and limited proteolysis to reveal how the phosphorylation and the BMH1 binding affect the activity of NTH1. H/D exchange and cross-

linking experiments coupled to mass spectrometry were used to determine the interacting surface of NTH1:BMH1.

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P20-42
The yeast multifunctional protein Hal3: still a source of surprises

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The *Saccharomyces cerevisiae* (*Sc*) Hal3 protein regulates cation homeostasis by inhibiting Ppz1,2 protein phosphatases. Recent work in our laboratory revealed that Hal3 is a multifunctional (moonlighting) protein, contributing with Vhs3 and Cab3 to an atypical heterotrimeric phosphopantothenoylcysteine decarboxylase (PPCDC), a key enzyme in CoA biosynthesis. In this complex, Hal3 and Vhs3 are functionally redundant, so the *cab3* or the *hal3 vhs3* mutants are not viable.

Sc Hal3 is composed of a ~250 aa core responsible for the PPCDC activity, plus an N-terminal extension and a ~60-residues highly acidic C-tail. The latter features are necessary for full inhibition of Ppz1. Analysis of fungal genomes reveals that the fission yeast *Schizosaccharomyces pombe* (Sp) contains a unique ORF (SPAC15E1.04) that could encode a Hal3 protein. Surprisingly, SPAC15E1.04 codes for a 625-residues protein which N-terminal half is similar to the *S. cerevisiae* Hal3 core (plus a short acidic tail) apparently fused to the only genomic sequence in *Sp* encoding the enzyme thymidylate synthase (TS). Resequencing of this genomic locus in *Sp* and *S. japonicus* confirmed the anomalous nature of these ORFs. Surprisingly, large-scale gene disruption analysis claims that the SPAC15E1.04 deletion is not lethal, in contrast to the prediction that both PPCDC and TS activities should be essential. To solve this puzzling problem, we have cloned and expressed the entire SPAC15E1.04 ORF, as well as the N-terminal (Hal3-related) and C-terminal (TS-related) halves, in *S. cerevisiae* and *E. coli*. We have demonstrated that the N-terminal half is able to inhibit *in vitro* Sc Ppz1 activity, as well as its *Sp* homolog Pzh1, and partially complements the cation-related phenotypes of the *Sc hal3* mutant. On the other hand, it provides PPCDC function, as it rescues lethality of the *cab3* and *hal3 vhs3* mutants. Recent evidence shows that SPAC15E1.04 is expressed as a single 70 kDa polypeptide under normal growth conditions and that its deletion is indeed lethal, in keeping with the predicted essential functions of the encoded proteins.

Both PPCDC and TS are highly conserved across the evolution, but this fusion polypeptide seems restricted to this specific Ascomycota class. Further work should reveal any possible evolutionary advantage derived from this surprising combination of essential proteins.

P20-43
Insights into the recognition of dynein light chain Tctex-1 of various cellular protein targets

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Dynein is a large cellular motor capable of transporting both organelles and protein cargoes in a retrograde manner along microtubules. Two of the dynein light chains, DLC8 and Tctex-1, are known to associate to the N-terminus of dynein intermediate chain and, presumably, interact with various protein targets that are to become transported. Despite the lack of protein homology, both DLC8 and Tctex-1 are homodimers that adopt a similar fold, with two alpha helices flanked by five β-sheets. In addition, one β-strand of each monomer becomes swapped in the other monomer, and, both in DLC8 and Tctex-1, the protein target binds forming an extended antiparallel β-strand that extends the preformed β-sheet. The sequence requirements necessary for DLC8 binding to protein targets is well known, with proteins stretches highly resembling either the KSTQT or KDTGIQVDR motifs. Conversely, little is known about the amino acid sequences that must be present in protein targets that bind to Tctex-1. Thus, we have systematically screened cellular proteins with the intention of mapping down the minimal Tctex-1 binding motif. Using pepscan, yeast two-hybrid and ITC the binding of Tctex-1 to cellular proteins CD5, DKK, Lfc, IC1, IC2, REIK, Orexin receptor and Rhodopsin has been characterized.

P20-44
PX family proteins at the interface between intracellular trafficking and signalling

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Following endocytosis, the fates of receptors, channels and other transmembrane proteins are determined via specific endosomal sorting pathways. These include lysosomal degradation and subsequent attenuation, or recycling to the cell surface for continued activity. Two distinct members of the phox-homology (PX) protein family, SNX17 and SNX27, are critical regulators of recycling from endosomes to the cell surface. We recently discovered that SNX17, SNX27 and a third homologue SNX31 all possess a novel structure similar to band4.1/ezrin/radixin/moesin (FERM) domains [1]. SNX17 has been shown to bind to NPxY sequences in the cytoplasmic tails of cargo notably the endothelial adhesion molecule P-selectin and the amyloid precursor protein (APP) central to Alzheimer's disease. We find that SNX17 and SNX27 display similar affinities for NPxY sorting motifs, indicating conserved functions in endosomal recycling for this protein family. Our ongoing work identifies novel impacts of these proteins on a wide range of signalling pathways. Firstly, we show for the first time that all three proteins are able to bind Ras GTPases through their FERM domains, and provide a structural model for their diverse interactions derived from X-ray crystallographic studies. Second, peptide array studies identify a role in trafficking of many other cargo molecules including GPCRs, growth factor receptors and integrins. PX-FERM proteins may then control endosomal trafficking of many classes of adhesion and signalling receptors, and potentially regulate spatially restricted Ras signalling cascades from intracellular organelles. Together, these molec-

ular interactions place the PX-FERM proteins at a hub of both endosomal sorting and signalling processes.

P20-45
Physicochemical investigation of a multistage DNA repair process

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Oxidation of DNA by reactive oxygen species generated during aerobic respiration is a major cause of genetic damage that can produce mutations and potentially lead to cancer and aging. A major DNA oxidation product is 8-oxoguanine (oxoG), a base with a high mutagenic potential. In bacteria, this lesion is repaired by formamidopyrimidine-DNA glycosylase (Fpg). We have attempted a comprehensive characterization of oxoG recognition by Fpg. First, we have obtained thermodynamic parameters for melting of DNA duplexes containing oxoG in all possible nucleotide contexts. The energy of stacking interactions of oxoG was in strict dependence on oxoG nucleotide environment, which may affect the recognition of damage and the efficiency of eversion of oxoG from DNA helix by Fpg. Next, we established how the flexibility of DNA context affects damage recognition by Fpg. Then, we have found that DNA containing oxoG next to a single strand break provides a good substrate for Fpg, as soon as all structural phosphate residues are maintained. Using site-directed mutagenesis, we have addressed the functions of many previously unstudied amino acid residuess that were predicted to be important for Fpg activity by molecular dynamics simulation and phylogenetic analysis. Of note, many substitutions abolished the excision of oxoG but did not affect the cleavage efficiency of abasic substrates. Finally, we investigated the contribution of separated structural domains of Fpg to specific enzyme-substrate interaction. Surprisingly, despite the absence of the catalytic domain, C-terminal domain of Fpg possessed a low residual ability to recognize and cleave abasic substrates. Our study sheds light on mechanism details of Fpg activity, with the ultimate goal of understanding how binding energy can be spent by Fpg for catalysis.

P20-46
Synchrotron radiation circular dichroism to study the binding of the DGAT1 peptides with substrates

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The bovine enzyme diacylglycerolacyltransferase1 (DGAT1) is a large transmembrane protein of unknown 3-D structure involved in the final step of triglycerides synthesis. In this study, peptides of different sizes corresponding to the putative binding sites of DGAT1 were synthesized to investigate their interaction with membrane bound molecules. Using Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy, the disordered structure of the peptides in aqueous solution was confirmed over their conventional CD spectra with minima at 198 nm, but gave an additional small positive band at 182 nm. Furthermore, an unordered to helix transition was observed in the binding to the substrates (dioleoylglycerol and oleoyl-CoA) when the two binding sites were together in the peptide sequence. Oriented CD was employed to determine the peptides orientation in membrane models and showed that the helical section of the DGAT1 peptides lies parallel to the membrane surface in the fully hydration state by the pronounced band at 207 nm.

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P20-47
Structural and thermodynamic analysis of the interactions between PPXY viral late domains and HNEDD4 third WW domain

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The interactions between viral late domains and their cellular targets are crucial for the completion of the life cycle of encapsulated viruses and have been propose as attractive targets for the development of broad spectrum antivirals. We present here a structural and thermodynamic analysis of the interactions between peptide ligands corresponding to the late domain sequences of ebola, HTLV-1, rabies and Marburg viruses and the third WW domain of the human ubiquitin ligase NEDD4, identified as the cellular target of PPXY type viral late domains. Our analysis has provided insight into the natire and magnitude of the forces that are driving these interactions, indicating that the same factors (conformational redistribution of both ligand and protein and water mediated interactions) identified for SH3 domain complexes, might be also of relevance for WW domains binding. Additionally, two different thermodynamic profiles have been observed, that point towards the existence of two different binding modes. This has been confirmed by the NMR solution structures of the HTLV-1 and EBOLA complexes with HNEDD4′s third WW domain.

P20-48
Non-canonical residues of the marginally stable monomeric ubiquitin conjugase from goldfish are involved in binding to the C terminus of Ring 1B

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E2 ubiquitin conjugases are ~20 kDa enzymes involved in ubiquitination processes in eukaryotes. The E2s are responsible for the transference of ubiquitin (Ub) to E3 enzymes, which finally transfer Ub to diverse target proteins, labelling them for degradation, localization and regulation. Although their functions are relatively well-characterized, their conformational stabilities are poorly known. In this work, we have used, as a model for our biophysical and binding studies, the E2-C from Carassius auratus (goldfish), a homologue of the human ubiquitin conjugase UbcH10. E2-C^{ca} was a monomeric protein with an elongated shape; moreover, the protein was only marginally stable within a narrow pH range (from 6.0 to 8.0). We also explored the binding of E2-C^{ca} towards non-canonical E3 ligases. Binding of E2-C^{ca} to the C terminus of murine Ring 1B (C-Ring1B), which does

not contain the RING finger of the whole Ring1B, occurred with an affinity of ~400 nM, as shown by fluorescence and ITC. Furthermore, binding of E2-C^{ca} to C-Ring1B did not occur at its canonical E2-loops, since residues M43 and F53, far away from those loops, were involved in binding. Thus, the C-Ring1B-interacting region of E2-C^{ca} comprises the first β-strand and nearby residues.

P20r-49
Molecular recognition mechanisms of microtubule plus end tracking proteins

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Microtubule (MT) plus-end tracking proteins (+TIPs) comprise a structurally and functionally diverse family of proteins that preferentially accumulate at MT growing ends. +TIPs form dynamic networks where most of the protein-protein interactions are mediated by End-Binding proteins (EBs). EBs were recently recognized to be master regulators of +TIP networks because: they are able to autonomously track MT tips and to recruit a large variety of other +TIPs to this location. EBs are modular proteins comprised of two functional domains connected by a long linker: a calponin-homology (CH) domain, responsible for MT binding, and a coiled-coil (CC) domain, responsible for partner binding. Here, we present structural and functional data describing how the concerted action of the CH, linker, and CC domains of EBs accounts for the autonomous MT tip tracking, regulation of MT dynamics, and recruitment of numerous partners to MT ends. Using a combination of structural and biochemical techniques, we propose a structural model for EB proteins in solution. Furthermore, our biochemical data suggest an important role for long-range electrostatic interactions between the CC domain of EBs and the MT lattice, which allows the EBs to discriminate between the MT-lattice and MT-tips. We also present insights into the molecular recognition mechanism of EBs by +TIPs containing a 'MT-tip localization signal' (MtLS), which comprises the short linear sequence motif SxIP. By means of an exhaustive functional analysis, we have derived the sequence determinants of a canonical MtLS and correlated the EB-binding and MT tip tracking activities of different SxIP-containing +TIPs. Moreover, we have investigated how phosphorylation regulates the EB-SxIP interaction and, consequently, MT-tip localization. Our data establish a favorable basis for computational approaches to search for novel TIPs in entire genomes.

P20-50
The structural rearrangements in fibri(noge)n fragment Bβ118-134 leading to the formation of neoantigenic determinant and site of protofibril lateral association

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Exposition of neoantigenic determinant (NAD) for mAb I-3c and a site of protofibril lateral association in fragment Bβ118-134 [Lugovskoi et al 2009] is a result of intermolecular rearrangement of the fibrin molecule, caused by FpA cleavage. This study presents the data about more precise NAD localization in Bβ118-134 and the mechanism of its exposition. Competative ELISA and turbidimetric analysis of the effects of peptides Bβ109–126 and Bβ121–138 on fibrin polymerization showed that only the latter inhibited the binding of mAb I-3c to fibrin and fibrin polymerization. Comparison of mAb I-3c binding to fibrins of different species by SPR and ELISA showed that mAb binds to human, horse and rabbit fibrin, but not to bovine or rat fibrin. Analysis of the fibrin amino acid sequences of Bβ118–138 of these species allows us to localize the NAD in fragment Bβ126–134 and suggest an important role of Lys130 in its formation. Comparative computational analysis of the interchain interactions in fibrinogen and fibrin showed that the probability of the chain interactions in β-α and β-γ pairs decreases by 14% and 15% respectively and between chains α and γ increases by 36%. We suggest that these structural rearrangements lead to NAD formation and provide fibrin molecules with the site of protofibril lateral association.

P20r-51
Conformational changes associated to the formation of DnaJ-DnaK complex

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DnaJ from *Escherichia coli* is a chaperone that belongs to the Hsp40 family. It acts as a cochaperone of DnaK, the main bacterial Hsp70, but it also exerts an independent chaperone activity on folded and unfolded client proteins. Here we study how complex formation with DnaJ modifies the conformation of a folded substrate, RepE, the replication initiation factor of plasmid F, and its functional consequences. DnaJ induces conformational changes in both domains of RepE, which include an increase in the intermonomeric distance within the RepE dimer and a conformational change in the C-terminal domain that stimulates binding to DNA. We show that the N-terminal domain of RepE is sufficient to form a stable complex with DnaJ, an identify an interaction with residues 51–55 located in a disordered loop at this domain as essential to fully induce the conformational changes in the client substrate. Analysis of the 3D volumes of DnaJ complexed to RepE and the deletion mutant RepE$_{1-144}$reveals that a large interaction interface is formed between both monomers of the chaperone and the substrates. They also show that the intrinsic flexibility of DnaJ might mediate the ability of the chaperone to bind substrate proteins of different size and/or conformational properties.

P20-52
Unraveling high zinc tolerance mechanisms: is citrate the Gordian knot?

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Zinc is an essential metal for all organisms, participating in the structure and/or function of many proteins. However, zinc excess is as deleterious to cells as zinc deficiency. Our group's research is centred on the homeostatic mechanisms induced by zinc excess in *Saccharomyces cerevisiae*. Hence, we reported how zinc overload results in a concomitant decrease of intracellular iron levels, as well as aconitase and cytochrome c activities in wild-type cells[1]. Besides, we isolated a deletant mutant (*pif1*D) exhibiting a clear high zinc-tolerance phenotype[2]. Pif1 is a DNA helicase

associated to the maintenanace of mitochondrial DNA (mtDNA), whose deficiency is complemented by Aco1 moonlighting DNA-binding function. Because of the accumulating evidence of a relation beween Aco1 protein/function and zinc and iron homeostasis, we decided to investigate the domino effect that would link all these factors. On the one hand, we examined the response to high zinc (zinc resistance and metal accumulation) of different *aco1* mutants[3], exhigiting distinct DNA binding and(or enzymatic activity alteration (*aco1*^{R476S}, *aco1*^{R668S}, *aco1*^{C448S} and *aco1*DD4). In addition, the effect of *ACO1* overexpression on zinc tolerance has been tested. On the other hand, since there is an effect of substrate accumulation (citrate) in cells where Aco1 activity is absent (TCA cycle arrest), and citrate is a natural chelator of iron-labile cell pool, we are investigating if the 'iron starvation phenotype' that we observe in D*aco1* and D*pif1* cells can be related to citrate/iron chelation, and subsequent inductions of the Fe operon machinery and iron accumulation. To this aim we are analyzing metal accumulation, zinc tolerance and citrate levels in D*pif1*, D*aco1*, D*cit1*[4], D*idh1*/*2*[4], D*cit1*D*aco1*[4], D*cit1*D*idh1*/*2*[4] mutnats grown on normal, Zn- or Fe- supplemented cultures.

Our hypothesis is that any molecular event leading to Aco1 loss of function will suppose an accumulation of citrate that would cause the drop of labile-iron cell contents, which would switch on the Fe operon, this leading to an increase of Fe levels that confer high zinc tolerance to cells. Therefore, our results suggest that this scenario shows the existing connection between metabolic pathways (TCA cycle), mtDNA stability, oxidative stress response and iro/zinc cell status.

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3. Kindly provided by late Prof. Butow's lab (University of Texas, Dallas, TX) (*aco1*^{R476S}, *aco1*^{R668S}), except *aco1*^{C448S} and *aco1*DD4 constructed in our lab.
4. Kindly provided by Prof. McAlister-Henn's lab (University of Texas, San Antonio, TX).

P20-53
Ca2+ influx and tyrosine kinases trigger adenylate cyclase toxin endocytosis. Activation of a repair-response in target cells

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Humans infected with *Bordetella pertussis*, the whooping cough bacterium, show evidence of impaired host defenses. This pathogenic bacterium produces a unique adenylyl cyclase toxin (ACT) which enters human phagocytes and catalyzes the unregulated formation of cAMP hampering important bactericidal functions of these immune cells that eventually, likely in synergy with its haemolytic capacity, causes cell death by apoptosis and/or necrosis. In a previous work we demonstrated that ACT is internalized into macrophages together with other membrane components, such as the toxin receptor, the integrin CD11b/CD18 (CR3), and GM1. The toxin-triggered internalisation occurs in these cells through two different routes of entry, a chlorpromazine-sensitive clathrin-mediated endocytosis and a clathrin-independent internalisation. An intracellular vesicular localization of ACT has also been observed in T cells by other authors. The goal of this study was to determine whether ACT uptake may take place into cells devoid of receptor. We present a detailed study addressing the endocytosis of ACT in three different cell lines: CHO cells, CHO-CR3 cells, in which the CR3 integrin has been stably transfected, and J774A.1(CR3-) macrophages, in which the intrinsic expression of CR3 has been knocked down. We show that ACT is rapidly eliminated from the cell membrane either in CR3-positive as negative cells by a calcium-mediated internalization. Activation of Src Tyr kinases and caveolin phosphorylation induced by ACT are other features of the endocytic strategies triggered. In addition, we find that the particular entry pathway followed by the toxin into the different cell lines might depend on the target cell physiology. Remarkably, we show for first time that cells injured by ACT are able to restore their membrane, suggesting that a repair response might be triggered by the target cell to protect it from the toxin injury.

P20r-54
Exploring the conformational changes in Inositol 1,3,4,5,6-pentakisphosphate 2-kinase

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Inositol polyphosphates are a wide group of second messengers, involved in key cellular events. In particular, IP_5 2-K converts inositol pentakisphosphate (IP_5) to inositol hexakisphosphate or phytic acid (IP_6) using ATP as phosphate donor. IP_6 is present in all eukaryote cells and plays an essential role in processes such as lymphocyte development or apoptosis. We are undertaken structural studies, by X-ray Crystallography, to understand the mechanism underlying the function and regulation of this key enzyme in cell biology. We have crystallized and solved the first structure of an IP_5 2-K, that from *A. thaliana*, always with inositide substrate or product in the active site. The enzyme is divided in two parts, N-lobe and C-lobe, the ATP being recognised by both. A large portion of the C-lobe forms a novel structural region (C_{IP}-lobe) in charged of the inositide recognition. Several observations, including the fact that the enzyme does not crystallize in absence of inositide, led us to conclude that IP_5 2-K undergoes important conformational changes to accomplish the catalytic cycle. A rationally designed mutant, W129A-IP_5 2-K, allowed the crystallization of the enzyme in absence of inositide for the first time. Concluding, we have been able to get three-dimensional structural information of the different conformational stages of IP_5 2-K adopted during catalysis (apo, nucleotide-bound and inositide-bound forms), which involves significant movements of the N-lobe. All these findings not only reveal a detailed mechanism of IP_5 2-K functioning but provide essential knowledge to design inhibitors for the enzyme.

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P20-55
A structural and kinetic study of Colchicine on glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*

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[1]Glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) is the first and rate-limiting enzyme of the pentose phosphate pathway. This pathway is responsible for the generation of NADPH and *de novo* production of cellular ribose-5-phosphate. Colchicine, an alkaloid obtained from *Colchicum autumnale,* is an effective and safe antifibrotic drug for the long-term treatment of gout, familial Mediterranean fever, amyloidosis and Behçet's disease. In this studywehave investigated the effects ofColchicine on G6PD from *Leuconostoc mesenteroides* and from baker's yeast (*Saccharomyces cerevisiae*). We have purchased *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* G6PD. We have chosen glucose-6-phosphate dehydrogenase because of the three important functions of the enzyme; first one is the rate-limiting property of the pathway and the second one is reduction of $NADP^+$ to $NADPH + H^+$, and the last one is ribose-5-phosphate synthesis which is essential for the *de novo* nucleic acid synthesis. The activity of the G6PD is measured by the increase in the different concentration of colchicine (0.010 – 0.100 mM). The obtained IC_{50}value of Colchicine is 0.077 ± 0.0056 mM for *Saccharomyces cerevisiae* G6PD and 0.063 ± 0.0057 mM for *Leuconostoc mesenteroides* enzyme. We have established that colchicine inhibits G6PD in a concentration dependent manner. Kinetic studies on the inhibition of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* G6PD by Colchicine have also been investigated by using Linewear-Burk double reciprocal plot and initial velocity data is analyzed. We have investigated the kinetic characterization, inhibition types and constants (Ki). The data were analyzed and the kinetic constants were calculated by means of a nonlinear curve-fitting program of the Statistica. We used molecular docking to investigate the probable binding sites of colchicine. And we have compared these results with our kinetic study results. Key words: G6PD, Colchicine, *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides*, inhibition, molecular docking.

P20-56
Solubilization of protein inclusion bodies by polyelectrolytes

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Protein aggregation is one of the most important problems in modern biochemistry, biotechnology and pharmacology. The appearance of the inclusion bodies (IB) in neuronal tissues could be the reason of rapid progressing of widespread neurodegenerative diseases such as Alzheimer or Parkinson diseases. The expression of eukaryotic protein in the prokaryotic cell (specifically hyperexpression of recombinant proteins) could led to misfolding and therefore to accumulation of synthesized protein in insoluble IB because of biochemical differences of translation systems or shortage of chaperonine. Protein in the IB is commonly inactive. Unfortunately, existing methods of extraction from IB (such as treatment by chaotropic agents or surfactants) require an individual approach for each recombinant protein and seldom result in height efficiency.

In this work the ability of synthetic polyanions to solubilize IB have been demonstrated for the first time. In comparison with chaotropic agents, extremely low concentration of polyelectrolyte is required for effective protein solubilization. Moreover, it has been shown that polysulfoanions are able to solubilize both amorphous and amyloid IB.

In addition, aninfluence of the type of charged group and hydrophobicity of polyelectrolyte on this process has been investigated. Increase of degree of polymerization has been shown to enhance the efficiency of IB solubilization. The probable mechanism of the observed process has been suggested.

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P20-57
Role of *Escherichia coli* CgtA in the stringent response and ribosome biogenesis

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CgtA is a conserved and essential small GTPase of the Obg family. In bacteria, it has been implicated in various growth processes, such as ribosome biogenesis, chromosome segregation, and the stringent response, but its precise function remains unknown. The subject of my PhD research is to understand the role of CgtA in stringent response and in ribosome biogenesis. The first part of my work consists in the characterization of *cgtA* expression. I showed that it is in operon with three genes coding for two ribosomal proteins and for a membrane protein of unknown function. Regulation of the operon expression was then studied using transcriptional fusion with GFP. This permitted to show that the operon is inhibited by ppGpp and DksA, hence linking its expression to growth rate and ribosome biogenesis control. (Paper 1, in preparation) The second part of my work aim to characterize the interaction between CgtA and RluD, a pseudouridine synthase involved in the maturation of the 23S RNA. Using bacterial two-hybrid, I studied the interaction between RluD and CgtA, and I mapped the domains involved in the interaction in the two proteins. Furthermore, using random mutagenesis, I was able to isolate mutations that abolish the CgtA/RluD interaction. These interaction mutants will be used to understand the role of the CgtA/RluD interaction in stringent response and in ribosome biogenesis. (paper 2, in preparation) In conclusion, the co-transcription of *cgtA* with ribosomal genes, its inhibition by ppGpp, and the CgtA/RluD interaction reinforces the role of CgtA in ribosome biogenesis. Therefore, we propose that the effects of CgtA on the stringent response might be indirect, as a consequence of ribosome malfunction.

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P20-58
Occurrence of a γ-glutamyltranferase-related enzyme in bovine spleen

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γ-Glutamyltranferase (γGT) is a key enzyme of the γ-glutamyl cycle, able to transfer the γ glutamyl moiety of glutathione (GSH) to acceptor molecules such as amino acids, peptides or simply to water. On the basis of molecular biology data only, the existence of another enzyme, distinct from γGT, capable of splitting the isopeptide bond of GSH, has been reported; this enzyme has been defined γGT-related (γGT-rel). γGT-rel has never been purified nor characterized, so that its existence as a functional enzyme, as well as its possible specific role in GSH homeostasis and hence in the mechanism responsible for the maintenance of cell redox status, are still to be assessed. A peculiar feature associated to γGT-rel is its strict specificity for GSH as substrate that appears to discriminate this enzyme from γGT. The latter is in fact able to act on a series of chromogenic synthetic substrates which are normally used to measure γGT activity in biological samples. Thus, taking advantage of an original specific γGT-rel assay combined with the well known chromogenic assay for γGT with γ-gluthamyl-p-nitroanilide as substrate, we were able to monitor changes in the relative specific activity (γ-glutamyl-p-nitroanilide/GSH activity) following some protein purification steps from bovine spleen. Results obtained are indicative of the actual presence of a GSH specific enzyme distinct from γGT, likely γGT-rel, whose activity is apparently elicited by incubation with oxidized glutathione.

P20-59
Lifespan extension and oxidative stress protective properties of NLaz depend on its ligand-binding ability

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NLaz (Neural Lazarillo) is a glycoprotein secreted mainly by neurons in *Drosophila*. As a member of the Lipocalin family, NLaz is expected to form an eight-strand b-barrel with a hydrophobic binding pocket. Despite being probably a key element for its function, no physiological ligand has been reported for NLaz. We show that NLaz binds *in vitro* ergosterol and 7(z)-tricosene in the micromolar range. A point mutation (L130R) in NLaz pocket compromises ligand binding. NLaz is involved in longevity by inhibition of the insulin signaling (IIS) pathway. Its human closest homologue, Apolipoprotein D (ApoD) shows a robust and evolutionarily conserved upregulation with age in the brain. NLaz loss-of-function (KO) fly mutants have reduced longevity and are hypersensitive to stress. Overexpressing NLaz rescues the NLaz-KO phenotype upon exposure to oxidative or desiccation-stress. However, a partial rescue is achieved by overexpressing the NLaz-L130R mutant. Moreover, NLaz-KO flies show altered mating behavior and a dramatically low courtship index. This phenotype is rescued by over-expression of NLaz-WT, but is partially rescued by the binding pocket-mutant NLaz-L130R. We conclude that NLaz ability to protect against oxidative stress and to regulate mating behavior is dependent on NLaz-ligand interactions. We are currently analyzing the expression profile in WT, KO and L130R NLaz flies. Genes involved in oxidative stress, metabolic responses including the IIS pathway, and pheromonal signaling will be explored in order to gain further insight into the importance of NLaz hydrophobic ligand-binding in the NLaz-dependent modulation of longevity and reproductive behaviors.

P20-60
Zinc induced folding is essential for TIM15 activity as an mtHsp70 chaperone

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The androgen receptor (AR) is a 919-residue hormone nuclear receptor responsible for the development of the male sexual phenotype. Its N-terminal transactivation domain (NTD) is intrinsically disordered and contains activation function 1 (AF1), the main activation function of AR, which is a potential therapeutic target for castration resistant prostate cancer (CRPC). However, due to its size and intrinsically disordered nature, this activation function has been little studied.We have studied the structural properties of the NTD of AR at atomic resolution using Nuclear Magnetic Resonance (NMR) spectroscopy.We have in addition studied the interaction of this domain to one of its cognate ligands, and observed how binding induces the formation of structure in this otherwise disordered domain. We have designed mutants to further characterize this interaction and its role in AR transcription activity in CRPC patients.

P20-61
CUG ambiguity in the structure of Ras1 and Cek1 from *Candida albicans*

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The polymorphic fungal pathogen *Candida albicans* has an ambiguous genetic code, as the universal leucine CUG codon is predominantly translated as serine (97%) but also as leucine (3%). Analysis of the rare *C. albicans* proteins containing CUG-encoded residues reveals that codon ambiguity shapes protein function and might have a pivotal role in signalling cascades associated with morphological changes and pathogenesis. The present study investigates the effect of leucine or serine at CUG positions (CUG ambiguity) in the structure and function of two key effectors of signalling cascades in *C. albicans*, Ras1 (GTPase) and Cek1 (protein kinase), which regulate the transcription of genes associated with morphological changes and pathogenesis. These two proteins contain a CUG residue in a strictly conserved and functionally relevant position. Synthetic genes coding for the active domains of Ras1 and Cek1 (serine and leucine variants for the CUG codon) were successfully cloned into expression vectors carrying different solubility partners. Furthermore, using an incomplete factorial approach, high level bacterial expression and purification protocols for the active domains of Ras1 and Cek1 were developed. Analytical size exclusion chromatography (SEC) and dynamic light scattering (DLS) results indicate that both recombinant proteins are monomeric. Crystallization screenings

with both proteins are currently in progress, aiming for the determination of their three-dimensional structures by X-ray crystallography. The structures of Ras1 and Cek1 with Ser or Leu at CUG positions, together with a thorough analysis of their stability and function *in vitro*, will provide valuable insights into a possible strategic role of natural codon ambiguity.

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P20-62
Biophysical study of the protein complex 14-3-3/ASK1

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ASK1 (Apoptosis signal-regulating kinase, MAP3K5) plays a critical role in the regulation of the apoptosis triggered by the cellular oxidative stress or immune response. Once activated, this homodimeric serine/threonine kinase phosphorylates MAP2K4/7 or MAP2K3/6 that in turn activate JNK or p38 signaling pathways. Diverse mechanisms such as phosphorylation, oligomerization and protein–protein interactions were shown to regulate the activity of ASK1. The 14-3-3 is the family of conserved regulatory proteins expressed in all eukaryotic cells [1]. They have been identified as a very important binding partners of ASK1. They bind to the phosphoserine motif at the C-terminus of the kinase domain and hold ASK1 in an inactive homodimeric state preventing the initiation of the signaling pathway. It has been shown that ASK1 is activated after dephosphorylation and dissociation of 14-3-3 in the presence of reactive oxygen species.

To understand the structural bases of ASK1 inhibition we decided to perform a structural study of 14-3-3/ASK1 complex. Here we report the preparation of the complex and its basic biophysical characterization. We optimized expression, purification and phosphorylation protocols of human recombinant enzymatically active catalytic domain of ASK1. Stoichiometry of the complex formed between ASK1 and 14-3-3 was studied using native gel electrophoresis and analytical ultracentrifugation.

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P20r-63
Structural and functional studies of FNE, a bacterial adhesion protein of *Streptococcus equi*: a 'Rebel' protein for crystallization

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Streptococcus equi is a gram-positive bacteria responsible for diseases of the upper respiratory tract in horses that can be fatal such as strangles in horses. Exceptionally, this bacterium is responsible for meningitis in humans. FNE is a protein involved in adhesion and virulence of *Streptococcus equi* by interacting with fibronectin at the extracellular matrix of the host cell. Fibronectin is a long-glycoprotein (250 kDa) organized into functional domains that interacts with different partners such as bacterial adhesion proteins. FNE Interacts with the Gelating Binding Domain of Fn (GBD), located near the N-terminus. We study the structural and functional aspects of this interaction as a model for bacterial adhesion to the GBD. We were unable to crystallize FNE or a truncated version lacking the disordered C-terminal peptide. We therefore developed artifical proteins, binding to FNE with the objective to create complexes of these proteins in complex with FNE and amenable to crystallization.We made use of a library coding for artificial protein constructed by repetition of a pattern designed HEAT from a thermophilic archaeal protein. Three artificial proteins interacting with the FNE have were obtained by phage-display and the corresponding complexes with FNE were tested for crystallization.

We will present this new innovative cristallogenèses technique and the structure of the complex FNE/partner artificial obtained 1.83A.

P20-64
Regioselectivity of flavoprotein-mediated aromatic hydroxylation

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Flavoprotein monooxygenases perform chemo-, regio- and enantioselective oxygenations of organic substrates. Reaction types catalyzed include hydroxylation, Baeyer-Villiger oxidations, *N*- and *S*-oxygenation, desulfurization, epoxidation and halogenation (1). Here we summarize new findings about the regioselectivity of flavoprotein-mediated aromatic hydroxylation.

FAD-dependent aromatic hydroxylases show diverse substrate binding pockets and selectively convert a wide range of natural and anthropogenic phenolics (2). An intriguing feature of these enzymes is that during catalysis, the flavin cofactor visits two active sites. To gain further insight into the action mechanism of flavoprotein aromatic hydroxylases, we have addressed the properties of 3-hydroxybenzoate 6-hydroxylase (3HB6H) from *Rhodococcus jostii* RHA1.

3HB6H is a homodimer that converts a restricted number of 3-hydroxybenzoate analogues into 2,5-dihydroxybenzoate derivatives by using NADH and oxygen (3,4). The crystal structure of 3HB6H reveals a bound lipid in a tunnel that connects the dimer interface with the active site. We hypothesize that the lipid guest protects the enzyme from oxidative stress.

Substrate docking and site-directed mutagenesis enabled us to identify residues involved in substrate binding and catalysis. We find that the substrate binding site of 3HB6H is a mirror image of the substrate binding site of sister enzyme 3-hydroxybenzoate 4-hydroxylase (3HB4H). However, different functions between critical residues exist.

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P20r-65
The M domain regulates the affinity of ClpB for nucleotides and substrate proteins

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ClpB is a bacterial ATP-dependent molecular chaperone that belongs to the Hsp100 family of ATPases associated with diverse cellular activities (AAA^+). This homohexameric chaperone solubilizes and refolds protein aggregates in cooperation with the DnaK (Hsp70) system. Each monomer contains two nucleotide binding domains (NBD) that display ATPase activity, and have to be coupled to exert the mechanical work necessary to extract unfolded monomers from the protein aggregate for their subsequent refolding. ClpB also contains a middle domain (M domain) located between the NBDs, which is essential for the chaperone activity of the protein and stabilizes the hexamer. We have studied the role of the M domain in the regulation of the ClpB affinity for nucleotides and substrate proteins. For this purpose, we have designed different variants of ClpB in which the M domain was partially or completely deleted. Besides these mutations, they also contained one or both NBDs unable to bind or hydrolyze ATP. Nucleotide and substrate binding data show that partial or complete deletion of this domain results in a reduced affinity for both ligands, and an enhanced ATPase activity of the protein. Proteolysis experiments also demonstrate that the M domain is essential to stabilize the NBD1 ring. Taken together, our results suggest that the M domain is involved in coupling the activity and conformational changes that take place in different regions of the chaperone to render it active.

P20-66
Characterization of a novel haloalkane dehalogenase DmxA from *Marinobacter* sp. ELB 17

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Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are microbial enzymes with catalytic activity for the hydrolytic conversion of xenobiotic and highly toxic halogenated aliphatic compounds to the corresponding alcohols (J. Damborsky et al, 2001, Janssen et al. 2005). These enzymes are able to convert a wide spectrum of substrates including halogenated alkanes, cycloalkanes, alkenes, ethers, alcohols, ketones, and cyclic dienes (E. Chovancova et al. 2007). To date, HLD activity is known for only about a dozen different proteins and it shows wide range of substrate specificities, however, with the difference between individual members of the α/β-hydrolase superfamily. A novel HLD were found in Gram negative psychrophilic bacteria: DpcA from *Psychrobacter cryohalolentis* K5 and DmxA from *Marinobacter* sp. ELB 17. DmxA enzyme was crystallized in the optimized condition and crystals of the enzyme were measured at X-ray detector Mar345 (IMG, ASCR Prague). Crystals diffracted to the resolution of 2.4Å. To observe the changing in the active site, the enzyme was crystallized with the ligand. Iwasaki method was used for measuring of the enzyme activity. Results for the native enzyme as well as for the crystals of HLD were obtained.

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P20-67
The ferritin-metallothionein connection: chemical implications in a biological scenario

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The physiological function/s of metallothioneins (MTs) is still unknown, although it has been suggested that they are involved in homeostasis of copper and zinc, global metal detoxification, as well as free radical scavenging.

Few examples are known of proteins interacting with ferritin (Ft), the iron-storing protein, which plays a crucial role in iron metabolism and is often observed at elevated levels in some radical-mediated diseases presumably devoted to sequester free iron. Literature data has shown that Fe cannot displace Zn from Zn-MT, but our recent studies [1] have shown that the interaction between Ft and the Zn-complexes of the mammalian MTs present in brain (MT1, MT2 & MT3) gives rise to a simultaneous Zn and Fe metal delivery that should be avoided in living systems to prevent the oxidative damage caused by the released Fe(II) ions. When Ft and MT interact, the Fe(III) is reduced and mobilized from the Ft cavity and the MT cysteine thiolates become fully oxidized to disulfide with the concomitant release of Zn(II). These results point out a so far unrecognized process by which proteins that independently bind metal ions to protect the cell against metal toxicity may cause damage if interacting when coming together.

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P20-68
Characterization of bovine lens aldose reductase enzyme and its kinetics study on cofactor NADPH

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Aldose reductase is a target enzyme of polyol pathway and it converts glucose to sorbitol in the presence of NADPH when high sugar concentration is presented in blood. Aldose reductase enzyme has huge risk factor for cataract and causes neuropathy and rethinopathy leading to irreversible damage for retina. In addition, sorbitol, sugar alcohol, collects in the cells, and leading to osmotic pressure and progressive opaqueness in lenses, and causes loss of vision. In this study, we examined aldose reductase enzyme related with cofactor NADPH and calculated its kinetic parameters. Moreover characterization of bovine lens aldose reductase enzyme was studied. For this aim, the lenses of bovine eyes were gotten from slaughter house and they were weighed for analysis. Then, homogenization and purification procedures were applied for obtaining of aldose reductase enzyme. Next, optimum pH, salt concentration ($LiSO_4$), suitable buffer system, storage and temperature conditions were examined for aldose reductase enzyme activity. While optimum pH of this enzyme was found as 7.6, optimum salt concentration, temperature and storage condition was observed as 270 mM, 25°C and 2 weeks, respectively. The effect of cofactor, NADPH, was carried out on bovine lens aldose reductase and The Michaelis- Menten and Lineweaver-Burk plots were drawn. As a result, V_{max} value for NADPH on bovine lens aldose reductase was found as 1.4 nmol/min/mg. Moreover, K_m value for NADPH was obtained as 35.81 μM.

P20-69
Snail1 and Snail2 bind and repress the human and mouse E-cadherin promoter through distinct combinations of zinc fingers

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Snail1 and Snail2 are two members of the Snail superfamily of transcriptional repressors with similar DNA binding structure, constituted in vertebrates by four and five C2H2 zinc fingers, respectively. Snail1 and Snail2 are presently considered key mediators of epithelial-to-mesenchymal transition (EMT) process in both development and tumor progression. Snail1/2 proteins act as transcriptional repressors that bind to a subset of E-box core motifs (CAGGTG) in target gene promoters, like the *E-cadherin* promoter. Nevertheless, Snail1 and Snail2 exhibit important differences in their binding affinity to *E-cadherin* promoter differentially target a subset of genes and play distinct roles in tumor progression. The molecular bases for such differences have not been yet defined. In particular, the role of the various zinc fingers of Snail1 and Snail2 in the binding to the E-boxes in target gene promoters, or the influence of different E-boxes, is presently unknown. We have investigated the involvement of the different Snail1 and Snail2 zinc fingers in the binding and repressor activity to the mouse and human *E-cadherin* promoters. The results obtained strongly suggest that the individual zinc fingers of Snail1 and Snail2 play distinct roles in the repressor activity of both factors, not all of them being equivalent neither essential. However, specific combinations of zinc fingers (ZF1/ZF2 of Snail1 or ZF3/ZF4 of Snail2) are required for a full repression depending also of the specific context of the E-boxes (i.e, mouse or human *E-cadherin* promoters). These results suggest that the architecture of the zinc fingers and/or the specific promoters might determine the specificity and affinity of Snail1/Snail2 factors to different gene targets.

P20-70
Structural basis for rat antiviral NK cell immune recognition

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Natural killer cells (NK cells) were discovered for their ability to spontaneously kill certain allogeneic tumour cell lines, without any previous sensitization. NK cells are part of non-adaptive immune response with very short reaction time against pathogens such as viruses, intracellular bacteria, parasites, and they are responsible for elimination of certain tumour cells and thus they are able to fight against malignancy and formation of metastasis. Activity of NK cells is regulated through the balance of activation and inhibitory signals mediated by corresponding NK cell surface receptors recognizing non-self ligands or markers of cell health, respectively.

We selected rat NK cell C-type lectin-like receptors as a model system for studying these processes on structural level. Rat inhibitory receptor NKR-P1B binds to a genetically linked ligand, protein Clrb, which serves as a marker of cell health and is rapidly downregulated during viral infection or stress response. Interestingly, NKR-P1B receptors from WAG and SD rat strains differ in only several amino acid residues, but these differences lead to an opposite outcome when WAG or SD rat strains are challenged by cytomegalovirus infection due to the difference in recognition of viral RCTL decoy ligand imitating Clrb.

We have expressed soluble dimeric forms of rat NKR-P1B SD and WAG receptors and their Clrb ligand by means of transient transfection of HEK293 cell line. While Clrb was successfuly crystallized, NKR-P1B SD crystals provide diffraction to 2.2 Å.

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P20-71
Features of homocysteinylated caseins: from spherical aggregates to amyloid fibrils

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Intrinsically disordered proteins (IDPs) belong to the most intriguing and mysterious 'grey figures' of current biology. Poorly studied due to the restrictions of traditional physico-chemical techniques, IDPs constitute a significant part of proteome and have numerous functions. Moreover, many disordered proteins are able to form compact structures upon minor changes in polypeptide chain properties, and also in complexes with their part-

ners. The main goal of the present work was to investigate the effect of homocysteinilation on the structure and properties of IDPs using dairy a_{S1}, b and k-caseins as model systems. Homocysteinilation is a post-translational covalent protein modification taking place upon the reaction of blood homocysteine thiolactone with free amino group of lysine residues. Therefore, homocysteinilation represents a convenient biochemical tool that can introduce point changes into protein structure on one hand, and on the other hand this process is of a great physiological significance and is involved into pathogenesis of neurodegenerative, cardiovascular and other diseases. Casein homocysteinylation leads in all cases to aggregation, confirmed by DLS and SDS-PAGE methods. Under a 100-fold excess homocysteine thiolactone a protein molecule was shown to incorporate an average of 1.5 homocysteine residues. The data acquired from various spectroscopy methods suggests that all three caseins undergo structural changes upon homocysteinilation, whereas the most interesting results were found in case of k-casein. In the present work we have shown that a prolonged κ-casein exposure to homocysteine thiolactone results in the emergence of a clearly defined Congo Red absorption maximum at 540 nm characteristic for the formation of amyloid structures. Fluorescent and electron microscopy revealed the formation of large spherical particles measuring 2–3 mm for homocysteinylated a_{S1} and b-caseins, whereas k-casein tends to form heterogeneous population of irregularly shaped aggregates composed of 200–300 nm long fibril bundles.

P20-72
Anion-transport blockers inhibit VDAC1 channel conductance, oligomerization and apoptosis

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Apoptotic signaling to the mitochondria results in the release of pro-apoptotic proteins, such as cytochrome *c* (Cyto *c*) from the intermembranal space to the cytosol, triggering caspases activation and apoptotic cell death. However, the mechanisms by which apoptotic initiators, including Cyto *c*, are released from mitochondria remain unclear. The Voltage-Dependent Anion Channel (VDAC), located in the outer mitochondrial membrane, serves as a controlled passage for adenine nucleotides, Ca^{2+} and other metabolites into and out of mitochondria, thus playing a crucial role in regulating the metabolic and energetic functions of mitochondria. It is accepted that VDAC1 has a key-role in apoptosis and is involved in the release of pro-apoptotic proteins, such as Cyto *c* and binds anti-apoptotic proteins. Recent studies from our lab demonstrated that VDAC1 oligomerization is coupled to Cyto *c* release and apoptotic cell death as induced by various stimuli. In this study, we demonstrate inhibition of apoptosis by inhibiting VDAC1 oligomerization. The effects of several known anion transport inhibitors, DIDS, SITS, H_2DIDS, DNDS and DPC, on apoptosis and VDAC1 oligomerization, as revealed by chemical cross-linking and immunoblotting or monitored in living cells by Bioluminescence Resonance Energy Transfer (BRET2) assay, were evaluated. All reagents inhibited both apoptosis and VDAC oligomerization as induced by various stimuli. We also demonstrate that these reagents interacted with bilayer-reconstituted VDAC1 and decreased its channel conductance, suggesting their direct interaction with VDAC1. The results clearly indicate VDAC1 to be a component of the apoptosis machinery and support the suggestion that VDAC1 oligomerization is coupled to apoptosis induction. Inhibiting VDAC1 oligomerization and thereby apoptosis, may offer a therapeutic strategy for neurodegenerative diseases such as Alzheimer's or Parkinson's disease, where apoptosis is highly activated.

P20-73
Characterization of the structural properties of the intrinsically disordered N-terminal transactivation domain of the androgen receptor

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The androgen receptor (AR) is a 919-residue hormone nuclear receptor responsible for the development of the male sexual phenotype. Its N-terminal transactivation domain (NTD) is intrinsically disordered and contains activation function 1 (AF1), the main activation function of AR, which is a potential therapeutic target for castration resistant prostate cancer (CRPC). However, due to its size and intrinsically disordered nature, this activation function has been little studied.We have studied the structural properties of the NTD of AR at atomic resolution using Nuclear Magnetic Resonance (NMR) spectroscopy.We have in addition studied the interaction of this domain to one of its cognate ligands, and observed how binding induces the formation of structure in this otherwise disordered domain. We have designed mutants to further characterize this interaction and its role in AR transcription activity in CRPC patients.

P20-74
Molecular insights into sector retinitis pigmentosa retinal disease

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Rhodopsin is the prototypical G-protein-coupled receptor located in the vertebrate retina and responsible for dim light vision. The receptor is constituted by a protein part, opsin, and the chromophore, 11-*cis*-retinal, covalently bound through a protonated Schiff base linkage to Lys296. Retinitis Pigmentosa (RP) is a group of inherited degenerative retinopathies genetically and clinically heterogeneous. Recently, the Asn55Lys mutation has been found to segregate with a mild form of sectorial RP. Sector RP is a puzzling atypical form of RP that is usually affecting only the eye fundus inferior quadrants. It is still unknown why the mutation affects only the lower section of the retina. In order to gain insights into the molecular aspects associated with these class of mutations, Asn55Lys was constructed by site-directed mutagenesis, expressed in COS-1 cells, immunopurified in detergent solution and studied by UV-visible and fluorescence spectroscopies, and radioactive binding assays. Asn55Lys showed a blue shift in the visible spectra and a distinctive behavior upon illumination when compared to WT. This suggests that the mutation influences the relative stability of the different intermediates formed upon photoactivation leading to a non-functional light-induced conformations. The lack of functionality was detected by a radioactive assay which showed that the mutant was unable to activate the G-protein transducin. Furthermore, fluorescence

spectroscopic analysis indicates that, after illumination, retinal would be temporary retained in the ligand binding pocket in contrast to WT rhodopsin. Overall, these findings may contribute to the knowledge of the molecular mechanisms underlying cell death associated with some RP mutations, like those causing sector RP.

P20-75
Chemical cross-linking and Hydrogen/Deuterium exchange as an alternative approach to studying the protein structure

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Determination of the three-dimensional structures of proteins has traditionally been realized by X-ray crystallography and NMR spectroscopy. Although these techniques provide high resolution atomic data, they have some limitations. Chemical cross-linking and Hydrogen/Deuterium (H/D) exchange combined with high resolution mass spectrometry offer an alternative approach to studying the protein structure. This method is fast, is general and uses small amounts of material. Our aim was to gain insight into structure of NKR-P1A and NKR-P1C protein, important activating receptors which play a key role in eliminating virally infected and tumor cells. We used homobifunctional cross-linking reagents DSS and DSG (amine–amine coupling) and heterobifunctional cross-linking reagent EDC (carboxyl–amine coupling). Mass spectrometry was used for cross-links identification and furthermore for precise revealing which residues were involved in the cross-link. The residues which were within a certain distance of each other were converted into covalent bonds by the cross-linking reagent and therefore provided distance constraints which were used for protein structure modeling. In the second approach, H/D exchange combined with mass spectrometry was applied to study the NKR-P1A loop conformation. The aim of this analysis was to compare the kinetics of H/D exchange for NKR-P1A and NKR-P1A in which the loop was removed and replaced with two alanines. H/D exchange revealed that the solution structure differs from the crystal structure in the conformation of the conserved loop. While the conserved loop is in close proximity to compact core in solution, it is extended from the core in the crystal structure where it interacts with the surface of a symmetry-related molecule. Finally, distance constraints derived from cross-linking and information on local solvent accessibility of proteins derived from H/D exchange have been implemented in the modeling of both receptors.

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P20-76
The oxidative protein folding process in mitochondria by NMR

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Several proteins of the mitochondrial intermembrane space are targeted by internal targeting signals. One of these proteins is COX17 with a-helical hairpin structure bridged by two intramolecular disulfides that is trapped by a Mia40-dependent oxidative process. We characterized the folding mechanism underpinning this process by an exhaustive structural characterization of COX17 in all stages and as a complex with Mia40. The two intramolecular disulfide bonds present in COX17 have different role in the folding mechanism of this protein. In order to clarify the role of the two intramolecular disulfide bridges, we solved the structures of two mutated forms with only one interhelical disulfide bond. Two consecutive induced folding steps are at the basis of the protein-trapping process. The subsequent step in the oxidative folding process is the transfer of a disulfide bond from MIA40 to the substrate. During the above mentioned step of the process MIA40 is reduced and it has to be regenerated to a functional state through the interaction with the flavin-dependent sulfhydryl oxidase ALR. The mechanistic basis of ALR–MIA40 interaction at atomic resolution has been characterized by biochemical and structural analyses of the mitochondrial ALR isoform and its covalent mixed disulfide intermediate with MIA40. This ALR isoform contains a folded FAD-binding domain at the C-terminus and an unstructured, flexible N-terminal domain. A specific region of the N-terminal domain guides the interaction with the MIA40 substrate binding cleft (mimicking the interaction of the substrate itself). The hydrophobicity-driven binding of this region ensures precise protein–protein recognition needed for an efficient electron transfer process. We dissected also the mechanism of the electron flux within ALR, characterizing at the atomic level the ALR intermediates that allow electrons to rapidly flow to cytochrome *c*.

P20-77
MCPIP1: regulating the regulator

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Inflammation is an extremely complex process evoked in the organism by harmful stimuli, including invading pathogens and tissue damage. The process is tightly controlled by numerous molecular mechanisms to provide the response optimized for the particular stimulant and to minimize the damage of organism's own tissues.

Among many proteins engaged in the regulation of inflammation one seem to be especially intriguing, as it is a multifunctional player negatively regulating inflammation-related processes. This protein, named MCPIP1, has been initially described as a pro-apoptotic transcription factor, then, as an ribonuclease degrading transcripts encoding proinflammatory cytokines, deubiquitinse inhibiting NF-κB and finally inhibitor of miRNA maturation. The activation of MCPIP1-coding gene is NF-κB- and Elk-1-dependent and the protein was shown to be phosphorylated, ubiquitinated and degraded rapidly following stimulation.

Currently we explore mechanisms of molecular regulation of MCPIP1. We discovered that the protein undergoes proteolytic cleavage and that one of the resulting proteins contains a CUE domain, known to bind ubiquitin moieties. Although ubiquitin binding was postulated to be essential for inhibition of NF-κB by MCPIP1 the deletion of CUE domain did not abolish this activity. However, MCPIP1 depleted of C-terminus-originating protein is devoid of the region responsible for homodimerization – the event crucial for its RNase properties. Therefore we postulate that proteolytic cleavage serves as a switch between diverse functions of MCPIP1 and is utilized to allow for fine and precise soothing of the response of activated cells. Here we present our latest results concerning this novel aspect of MCPIP1 functioning.

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P20-78
The structural basis for autonomous dimerization of the pre-T-cell antigen receptor

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β-selection, survival and proliferation of CD4-CD8- double-negative thymocytes, and subsequent αβ T-cell lineage differentiation. Whereas αβTCR ligation by the peptide-loaded major histocompatibility complex initiates T-cell signalling, pre-TCR-induced signalling occurs by means of a ligand-independent dimerization event. The pre-TCR comprises an invariant α-chain (pre-Tα) that pairs with any TCR β-chain (TCRβ) following successful TCR β-gene rearrangement. Here we provide the basis of pre-Tα–TCRβ assembly and pre-TCR dimerization. The pre-Tα chain comprised a single immunoglobulin-like domain that is structurally distinct from the constant (C) domain of the TCR α-chain; nevertheless, the mode of association between pre-Tα and TCRβ mirrored that mediated by the Cα–Cβ domains of the αβTCR. The pre-TCR had a propensity to dimerize in solution, and the molecular envelope of the pre-TCR dimer correlated well with the observed head-to-tail pre-TCR dimer. This mode of pre-TCR dimerization enabled the pre-Tα domain to interact with the variable (V) β domain through residues that are highly conserved across the Vβ and joining (J) β gene families, thus mimicking the interactions at the core of the αβTCR's Vα–Vβ interface. Disruption of this pre-Tα–Vβ dimer interface abrogated pre-TCR dimerization in solution and impaired pre-TCR expression on the cell surface. Accordingly, we provide a mechanism of pre-TCR self-association that allows the pre-Tα chain to simultaneously 'sample' the correct folding of both the V and C domains of any TCR β-chain, regardless of its ultimate specificity, which represents a critical checkpoint in T-cell development. This unusual dual-chaperone-like sensing function of pre-Tα represents a unique mechanism in nature whereby developmental quality control regulates the expression and signalling of an integral membrane receptor complex.

P20-79
X-ray structure of L-ribose isomerase in complex with L-ribose and a deduced catalytic mechanism

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L-Ribose isomerase catalyzes the reversible aldose-ketose isomerization between L-ribose and L-ribulose. L-Ribose has a potential usage as a precursor for the synthesis of L-nucleoside analogues, which are widely used as pharmaceutical compounds including anticancer drugs. An interest in its production is growing, but L-ribose is one of rare sugars, which are scanty monosaccharide in nature.

For the effective production and the physiological study of rare sugars, various enzymes for rare sugar production have been reported [1-3]. L-Ribose isomerase from *Acinetobacter* sp. (L-RI) is also one of the target enzymes [4]. In particular, L-RI has no similarity with known amino acid sequences of PDB, and an expected unique substrate recognition and its catalytic mechanism have been great interest. In our previous study, we reported the overexpression, purification and crystallization study of recombinant His-tagged L-RI [5]. Although we determined the overall structure of L-RI by using selenomethionine-substituted L-RI and found metal ion coordinated with three His and one Glu in putative active site, it contained a highly disordered region which was partly invisible. To understand the substrate recognition mechanism of L-RI, the complex structure with substrate is essential. Here we determined the crystal structure of His-tagged L-RI in complex with L-ribose. On the basis of the current structures in complex with substrates, we propose that L-RI likely adopts cis-enediol mechanism.

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P20-80
Study of the interaction between acriflavine and beef liver catalase by spectroscopic methods

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Acriflavine (3,6-diamino 10-methylacridinium chloride) is a bacteriostatic, fungicide, and trypanocidal agent causing both apoptosis and necrosis in the yeast Candida utilis. Interaction of the compound with catalase, an ubiquitous antioxidant enzyme, was assessed by electronic absorption, fluorescence, and circular dichroism (CD) spectroscopy of beef liver catalase (BLC) preincubated for increasing intervals with various amounts of acriflavine in phosphate buffer 0.1 M (pH 7.0) at 25°C. Incubation of BLC with increasing acriflavine concentrations led to hypochromicity and blue shift (up to 18 nm) at 404 nm (Soret absorption band maximum), and hypochromicity and red shift (up to 16 nm) at 275 nm (absorption band due to amino acids aromatic side chain). Based on spectrophotometric data, one apparent dissociation constant (K_d) was found, implying the presence of one site or several independent sites in BLC for acriflavine binding; K_d value decreased by a factor of 5 as preincubation went from 0 to 120 min. Fluorescence measurements showed that acriflavine quenched BLC intrinsic fluorescence via a static mechanism; a binding constant of 2×10^{10} M^{-1} was calculated for the BLC-acriflavine complex. Far-UV CD studies indicated that acriflavine caused alterations in BLC conformation; decrease in alpha helix,

and increase in beta sheet and random coils content, suggested partial unfolding of BLC secondary structure. In conclusion, the present *in vitro* study showed that acriflavine formed a relatively stable complex with BLC, altering the enzyme's structure. The negative enthalpy and positive entropy changes obtained from fluorescence studies revealed that hydrogen binding and hydrophobic interactions are predominantly involved in the interaction of acriflavine with BLC.

P20m-81
Kinetic mechanism and biochemical characterization of recombinant sucrose synthase isoform 4 from potato (*Solanum tuberosum* L.)

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Sucrose synthase (SuSy) is a retaining glycosyltransferase that catalyses the reversible conversion of fructose and a nucleoside diphosphate glucose into the corresponding nucleoside diphosphate and sucrose. The purpose of this work was to establish the kinetic and chemical reaction mechanism of SuSy in both sucrose synthesis and cleavage direction.

Primary plot analysis and product inhibition studies showed that a kinetic ordered *bi-bi* mechanism was followed, with UDPG binding first and UDP dissociating last from the enzyme. In the sucrose synthesis direction the kinetic constants were K_m (mM ± SE) for UDPG, 0.069 ± 0.009; for fructose, 17.68 ± 0.45 and k_{cat} (s^{-1} ± SE) for UDPG, 2.63×10^5; for fructose, 5.43×10^5. Besides, in the sucrose cleavage direction the kinetic constants were K_m (mM ± SE) for UDP, 0.075 ± 0.007; for sucrose, 24.63 ± 0.42 and k_{cat} (s^{-1} ± SE) for UDP, 1.10×10^5; for sucrose, 9.48×10^4. Product inhibition studies showed that UDP was a competitive inhibitor with respect to UDPG, K_{ic} = 145 ± 48 μM, and no inhibitor with respect to fructose. Sucrose was an uncompetitive inhibitor with respect to UDPG, K_{iu} = 32 ± 9 μM, and noncompetitive with respect to fructose, K_{inc} = 25 ± 1 μM.

From V_{max} versus pH plots of the sucrose synthesis reaction, a pK_a value of 7.3 of a single ionisable group in the UDPG-enzyme complex, which influences enzyme catalytic activity, was determined. However, in the sucrose cleavage direction two ionisable groups with pK_a′s of 5.3 and 8.7 were observed. In addition, the corresponding pK_a′s of the free enzyme were 5.7 in the sucrose synthesis direction, and 7.3 and 8.0 in the sucrose cleavage direction. These results will be discussed in the context of the chemical reaction mechanism of SuSy.

P20-82
The disordered N-terminal region of dengue virus capsid protein contains a drug targetable lipid droplet-binding motif

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Dengue is the major arthropod-borne human viral disease, for which no vaccine or specific treatment are available. We used nuclear magnetic resonance, zeta potential measurements and atomic force microscopy to study the structural features of the interaction between dengue virus capsid (C) protein and lipid droplets (LDs), organelles crucial for infectious particles formation. C protein binding sites to LD were mapped, revealing a new function for a conserved segment in the N-terminal disordered region, and indicating that conformational selection is involved in recognition. The results suggest that C protein positively-charged N-terminal region prompts the interaction with negatively-charged LDs, after which a conformational rearrangement enables the access of the central hydrophobic patch to LD surface. Altogether, the results allowed the design of a peptide with inhibitory activity of C protein-LD binding, paving the way for new drug development approaches against dengue.

P20-83
Characterization of Pdx-P450cam complex by paramagnetic NMR

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Bacterial cytochrome P450cam is a heme containing enzyme, participating in the hydroxylation of camphor to 5-exo-hydroxycamphor. Interestingly, the catalytic activity of P450cam is triggered by the interaction with the electron donor protein, putidaredoxin (Pdx). It has been speculated that Pdx binding induces the conformation change in P450cam, which not only optimizes the hydroxylation reaction itself but also minimizes the displacement of substrate from active site. Although a number of studies emphasize the significance of Pdx effector activity, the structure of the Pdx-P450cam complex has not yet been elucidated because co-crystallization of the proteins has failed so far.

We approach the molecular details of Pdx-P450cam interaction by paramagnetic NMR methods. By attaching paramagnetic NMR probes on P450cam surface, we were able to calculate the distances between P450cam and Pdx. Based on those distant restraints, the Pdx-P450cam complex structure was generated. The orientation of Pdx is nearly 180° opposite to earlier models. The structural analogue, the Adx-P450scc complex has been recently solved by X-ray crystallography. In this talk, the similarities and differences with our Pdx-P450cam model will be discussed.'

P20-84
Antizyme binding element of ornithine decarboxylase: analysis of critical residues

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Polyamines (PA) are small cationic molecules implicated in cell growth, proliferation and differentiation. Their intracellular levels are tightly regulated through the control of both ornithine decarboxylase (ODC), the key biosyntethic enzyme that converts ornithine into putrescine, and polyamine uptake. In fact, high PA levels induce the expression of antizymes (AZs), small proteins that bind to ODC and accelerate the degradation of the enzyme by the 26S proteasome, in an ubiquitin-independent manner, and also inhibit polyamine transport. Whereas mammalian ODC has a very short half-life, the trypanosomal enzyme is more stable, this discrepancy being mainly related to that trypanosomal ODC

does not interact with AZs. Early studies using chimerical mouse-trypanosome ODC proteins identified the locus responsible for the binding of ODC to AZs. This region, located between residues 110–145, is known as antizyme binding element (AZBE). The aim of our study was to identify the key residues involved in the interaction of ODC with AZs. For that purpose, multi-alignment analysis of the AZBE sequence of different ODC orthologs, and comparison with the equivalent region of antizyme inhibitors (AZINs), homologous proteins of ODC devoid of enzymatic activity, but interacting with AZs, led to identify five invariant residues (K115, A123, E138, L139 and K141). By using site directed mutagenesis and transient transfection essays, we analyzed the role of these residues on ODC activity and ODC-AZ interactions. In addition, the influence of the non-conserved residues between the mouse and trypanosomal enzymes in the AZBE region on ODC activity and stability was studied.

P20-85
Modulation of *Pyrococcus abyssi* NucS activity by the PCNA at functional and structural levels

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Pyrococcus abyssi NucS is a founding member of the novel endonuclease family that interacts with the replication clamp PCNA [1,2]. In *P. abyssi*, a hyperthemophilic archaeon, NucS and PCNA form a highly stable complex that can be isolated using gel filtration chromatography. Using a combination of small angle X-ray scattering and surface plasmon resonance analyses, we demonstrate the formation of a stable complex in solution, in which one molecule of the NucS homodimer binds to the outside surface of the PCNA homotrimer. We have investigated binding of the NucS-PCNA complex to branched DNA substrates using fluorescence anisotropy and fluorescence resonance energy transfer (FRET) techniques. Anisotropy experiments demonstrated high specificity of NucS for ssDNA. Moreover, our results reveal that PCNA is required for loading of NucS onto 5′ and 3′ ssDNA flaps embedded in the duplex regions. We have not observed preferential association of NucS with 5′ or with 3′ flaps; this is a rare characteristic for enzymes acting on DNA. Biochemical experiments also suggest that PCNA modulates NucS activity in several ways based on the ratio between the two proteins and on the substrate structures. However, higher levels of PCNA enhance the direct cleavage at the ss/dsDNA junction in both 5′ and 3′ flap. Finally, FRET measurements using double-labelled DNA flaps indicated that the complex formation decreased the end-to-end DNA distance of the DNA substrates used, thus indicating a marked DNA bending brought about by binding of the complex onto DNA. Our results indicate that the presence of a single major contact between the NucS and PCNA proteins, together with the complex-induced DNA bending, facilitate conformational flexibility required for specific cleavage at the ss/ds junction.

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P20-86
Functional dynamics in the voltage-dependent anion channel

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The voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, acts as a gatekeeper for the entry and exit of mitochondrial metabolites and controls cell life and death [1, 2]. Here we reveal functional dynamics of isoform one of VDAC (VDAC1) by a combination of solution NMR spectroscopy, Gaussian network model analysis and molecular dynamics simulation [3]. Micro- to millisecond dynamics are significantly increased for the N-terminal six β-strands of VDAC1 in micellar solution, in agreement with increased B-factors observed in the same region in the bicellar crystal structure of VDAC1. Molecular dynamics simulations reveal that a charge on the membrane-facing glutamic acid 73 (E73) accounts for the elevation of N-terminal protein dynamics as well as a thinning of the nearby membrane. Mutation or chemical modification of E73 strongly reduces the micro- to millisecond dynamics in solution. Since E73 is necessary for hexokinase-I-induced VDAC channel closure and inhibition of apoptosis [4], our results imply that micro- to millisecond dynamics in the N-terminal part of the barrel are essential for VDAC interaction and gating.

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P20-87
Oligomerization and specificity of yeast invertases are modulated by its non-catalytic domain

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Fructans and fructooligosaccharides are β-D-fructose based carbohydrates often linked to a sucrose skeleton, which are synthesized by 15–20% of the flowering plants and also by a number of bacteria and fungi. These compounds have been related to carbohydrate storage and processes of drought and freezing tolerance in plants. In addition, FOS are considered prebiotics since they selectively stimulate the growth/activity of *Lactobacilli* and *Bifidobacteria* from the animal digestive tract and exert a beneficial effect on human health, contributing to the prevention of cardiovascular diseases, colon cancer or osteoporosis. Therefore, the enzymes involved in fructans processing attract great biotechnological attention for the production of functional foods and pharmaceuticals. In particular, a detailed knowledge of the molecular mechanisms involved in substrate recognition, transfructosylating efficiency and product specificity of the enzymes used as catalyst for these processes is essential. We have solved the crystal structure of two invertases from yeast. First, the *Schwanniomyces occidentalis* Invertase, complexed with long substrates, revealed for the first time that the ancillary domain plays a direct role in oligomerization and substrate binding [1, 2], which is a unique feature that shed light on the molecular mechanism regulating specificity within the GH32 enzymes from

eukariota. We report also the *Saccharomyces cerevisiae* Invertase, an enzyme reported to adopt different aggregation states upon changes in the environment. The crystal structure revealed a sophisticated mechanism of molecular interaction between subunits that form higher aggregates throughout further involvement of the ancillary domains. Our results assign a direct catalytic role to the supplementary ß-sandwich domain of these enzymes, the first time that such a role has been observed within GH32 enzymes.

P20-88
Biology of cofactor F420 in mycobacteria

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The flavin cofactor F_{420} is believed to have an important role in the oxidoreductive reactions and anaerobic survival of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis. We have been working on a number of F_{420}-binding proteins in order to clarify the biochemical/physiological function of this cofactor in *Mtb*. In the absence of a commercial source for F_{420}, the co–expression of three proteins in the F_{420} biosynthetic pathway has enabled us to achieve F_{420} production levels of up to 10 times higher compared with the wild type *M. smegmatis* strain, providing us with high yields of F_{420} for our research.

Using X-ray crystallography, we have solved the first crystal structure of an F_{420}-dependent protein from mycobacteria, called F_{420}-dependent glucose-6-phosphate dehydrogenase (FGD1). FGD1 is the only known enzyme dedicated to the reduction of F_{420} in mycobacterial cells, providing the reduced cofactor for $F_{420}H_2$–dependent enzymes and possibly for other unknown metabolic needs. In *Mtb*, FGD1 also provides reduced F_{420} to an accessory protein (Ddn) for the *in vivo* activation of the nitroimidazopyran prodrug PA-824, currently in the Phase 2 clinical trials for anti-tuberculosis therapy against both replicating and persistent bacteria. We established that this activation is specific to *Mtb* and does not occur in human liver.

We also have shown that in pathogenic mycobacteria the cofactor F_{420}, while bound to another protein (Rv0132c), is translocated across the cytoplasmic membrane *via* the Tat pathway. This translocation may have evolved in mycobacteria to enable F_{420} utilization for metabolic or biosynthetic processes in the dense, lipid–rich cell wall, implying that the presence of F_{420} outside the cytosol is important for mycobacterial pathogenesis.

P20-89
Features of halorhodopsin-carotenoid complex from *Natronomonas pharaonis* membrane in the solubilized system

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Halorhodopsin is a retinal protein with a seven-transmembrane helix and acts as an inward light-driven Cl^- pump. In this study, structural state of the solubilized halorhodopsin (NpHR) from the biomembrane of mutant strain KM-1 of *Natronomonas pharaonis* in non-ionic detergent was investigated. A gel filtration chromatography monitored absorbances at 280, 504 nm corresponding to the protein and a lipid soluble pigment of bacterioruberin (BR) respectively has clearly detected an oligomer formation of the NpHRs and a complex formation between the NpHR and BR in the solubilized system. A molar ratio of NpHR:BR in the solubilized complex was close to 1:1. Further SDS-PAGE analysis of the solubilized NpHR cross-linked by 1%-glutaraldehyde has revealed that the NpHR forms homotrimer in detergent system. Although this trimeric structure was stable in the presence of NaCl, it was dissociated to the monomer by the heat treatment at 45°C in the desalted condition. Interestingly, the trimer dissociation on the NpHR was accompanied by the complete dissociation of the BR molecule from the protein, indicated that the cavity formed by the NpHR protomers in the trimeric conformation is important for tight binding of the BR. Because the binding affinity for Cl^- and the resistance to hydroxylamine under light illumination showed only minor differences between the NpHR in the solubilized state and that on the biomembrane, the influences of solubilization to the tertiary structure and function of the protein are thought to be minor. This NpHR-BR complex in the solubilized system has a potential to be a good model system to investigate the intermolecular interaction between the membrane protein and lipid.

P20-90
Interaction pathways control the substrate specificity in a β-glucosidase

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The β-glycosidase Sfβgly was submitted to random mutagenesis and the resulting library was screened searching for mutations that change the substrate specificity of Sfβgly. Eight of the selected mutations were placed at the C-terminal half of Sfβgly, whereas only two mutations occurred in the active site. The mutant and wild-type Sfβgly are similarly folded as evidenced by thermal inactivation and fluorescence spectra. The selected mutants were produced individually as recombinant proteins in *E. coli* BL21DE3 and purified. The changes on their preference for the substrate glycone were confirmed by the determination of enzyme kinetic parameters. Seeking for an explanation for the effect of these mutations, which were placed far from the Sfβgly active site, but changed its substrate specificity, contact maps based on the tertiary structure of Sfβgly revealed interaction pathways that link the point of the mutations to the active site residues involved in substrate binding and catalysis. In agreement, mutations which were selected independently mapped on the same contact pathway. As an additional test, site-directed mutants containing replacements at residues of the same interaction pathway were produced as recombinant proteins and purified. The kinetic characterization of these mutants indicated that mutations at the same interaction pathway caused similar effects on the substrate specificity. In brief, these data suggest that interaction pathways control the substrate specificity in Sfβgly.

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P20-91
Cysteine-rich insect toxins from *Lachesana tarabaevi* spider venom

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Venom of *Lachesana tarabaevi* (Zodariidae, ant spiders) exhibits high insect toxicity and serves a rich source of potential insecticides. Five new peptide insectotoxins named latartoxins (LtTx) were isolated from the venom using liquid chromatography. Complete amino acid sequences of LtTx (60–71 residues) were established by a combination of Edman sequencing, mass spectrometry and enzymatic and chemical proteolysis. Three toxins

have eight cysteine residues that form four intramolecular disulfide bridges, and two other molecules contain an additional cystine. Three LtTx are C-terminally amidated. LtTx can be allocated to two groups with members similar to CSTX and LSTX toxins from *Cupiennius salei* (Ctenidae) and *Lycosa singoriensis* (Lycosidae). The interesting features of the new toxins are the cysteine-rich region as in many neurotoxins from spider venoms and the linear part alike some cytolytic peptides. The C-terminal linear fragment (21 residues) of the most abundant peptide, LtTx-1a, was chemically synthesized, and its lytic activity was characterized on different bacterial strains and lipid vesicles. We suggest a membrane-dependent mode of action for LtTx with their linear parts acting as anchoring devices.

P20-92
Mapping of the integrin α6β4 binding site in the N-terminal region of the bullous pemphigoid antigen 1, BPAG1e

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Hemidesmosomes (HDs) are multiprotein complexes that mediate firm adhesion of epithelial cells to the underlying basement membrane [1]. The 230-kDa bullous pemphigoid autoantigen (BP230/BPAG1e) and the integrin α6β4 (a laminin receptor) are components of the HDs. BPAG1e belongs to the plakin family of cytolinkers that associate with and cross-link components of the various cytoskeletal systems. BPAG1e contains in its N-terminal region a plakin domain (a sequence of ~ 1000 residues conserved in the plakin family) formed by eight Spectrin Repeats (SR2 to SR9) and a SH3 domain inserted in the SR5. The N-terminal region of BPAG1e is important for its recruitment into HDs as it associates with the cytoplasmic region of the β4 subunit of the integrin α6β4 [2].

It has previously been shown by yeast two hybrid experiments that binding of BPAG1e to integrin β4 relies on the sequence which corresponds to the N-terminal tail that preceeds the SR2, and part of the SR2 [3]. In order to determine the affinity for β4 of the different fragments of BPAG1e we have developed an assay based on measuring changes in the fluorescence anisotropy of Oregon Green or Fluorescein-labeled BPAG1e proteins. The binding site has been located in a 18-residues long sequence of the N-terminal tail.

In addition to this, we have designed a collection of BPAG1e fragments corresponding to the SR2 and the 55-residues long N-terminal tail leading up to the SR2, and shorter versions that contain N-terminal deletions up to the beginning of the SR2. The recombinant proteins were expressed in *E. coli*. Here we report the crystal structure of the SR2 of BPAG1e. The structure has been solved by molecular replacement and was refined against data to 2.0 Å resolution. The SR2 structure is built up of three α-helices connected by short loops. Comparative analysis by circular dichroism of the SR2 and longer fragments that contain increasing sequences of the N-terminal tail suggests that the tail is an intrinsically disordered region.

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P20-93
Structural basis of specificity in Phaffia rhodozyma β-fructofuranosidase, a GH32 neo-FOS producing enzyme

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Several epidemiological studies have shown that food plays a key role in human health. Therefore, the food industry has been focusing on the design of new products with beneficial effects on heath, known as 'functional foods'. Many of these have an important ingredient in their composition called prebiotic, a selectively fermented component that causes specific changes both in composition and activity in the intestinal microflora that confer health benefits. Within prebiotics, fructans (which include fructooligosaccharides, FOS) are the most widely used compounds. Research on methods of producing these oligosaccharides involves both, the study of new compounds with novel functional properties, as well as finding more efficient ways of producing them. We report here the crystal structure of Phaffia rhodozyma β-fructofuranosidase (Ffase) complexed with a transfructosylating product that mimics the neo-FOS activity of this enzyme, from which a model for the mechanism of the Ffase transfructosylation reaction is proposed. This result contribute to a better understanding of the molecular basis regulating specificity among GH32 family members, which represent an interesting target for rational design of enzymes, showing redesigned activities to produce tailor-made fructooligosaccharides.

P20-94
Giant oligomeric extracellular *Glossoscolex paulistus* hemoglobin: stability and dissociation model, in the presence of urea, as evaluated by small angle X-ray scattering (SAXS)

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Giant extracellular hemoglobins, also known as erythrocruorins, have been investigated as a model of extreme complexity in oxygen-binding heme proteins. The extracellular hemogobin of *Glossoscolex paulistus* (HbGp) has a molecular mass of 3.6 MDa, determined by analytical ultracentrifugation (AUC). In this work, studies were performed, by small angle X-ray scattering (SAXS), aiming to contribute to understanding the urea effects on the HbGp oligomeric structure and stability. Oxy- and cyanomet-HbGp forms were investigated, at several urea concentrations, at pH 7.0 and 20°C, at protein concentrations of 0.5 and 3.0 mg/ml. SAXS data show that the cyanomet-HbGp is more stable than oxy-HbGp, and both forms undergo unfolding above 4.0–5.0 M of urea. Oxy- and cyanomet- forms, in the absence of the urea, present identical SAXS curves with R_g, D_{max} and I_0 values of 106 ± 1Å, 300Å, and 0.26 ± 0.01, independent of the protein concentration. In the presence of urea, up to 4.0 M of denaturant, no significant changes are observed in the values for the two parameters characterizing the scattering particle dimensions. However, the scattering intensity I_o is reduced from 0.26 to 0.10. In the higher urea concentration range, above 4.0 M, oxy-HbGp is dissociated with a considerable fraction of denatured protein.

Above 6 M of denaturant, and independently of protein concentration for both forms, irreversible dissociation and denaturation processes are observed, with a increase of R_g, D_{max} and I(0). Our results are consistent and support previous studies on the effect of urea upon three HbGp forms, monitored by various spectroscopic techniques, suggesting that HbGp undergoes oligomeric dissociation followed by denaturation at higher urea concentrations. In summary, HbGp unfolding process, in the presence of urea, is composed of two phases. The first one is associated to the protein oligomeric dissociation, while the second one to the subunits denaturation. Thus, the several dissociated species observed in solution, as a function of urea concentration, are assigned to HbGp intermediate state in the unfolding process.

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P20-95
Towards the structure of lymphocyte receptor hLLT1

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Natural killer (NK) cells are an intensively studied part of immune system, possessing unique ability to recognize and induce death of tumor and virus-infected cells without prior antigen sensitization. Their function is regulated by a fine balance of signals induced by multiple activating and inhibitory cell surface receptors and their interaction with the ligands present on the target cell. Recent research in their C-type lectin-like receptors repertoire has shown that ligands of some of these previously orphan receptors lie within their own family, describing a lectin-lectin interaction. This is the case of human inhibitory receptor NKRP1 (gene KLRB1) and its ligand LLT1 (gene CLEC2D). Previous studies have shown that overproduction of LLT1 in cancer cells or lower production of NKRP1 in NK cells is connected to cancerous manifestations. Our previous efforts to study this system on a structural level via recombinant expression in *E. coli* have shown that the proteins aggregate to inclusion bodies and their refolding was inefficient. We decided to try mammalian expression system based on transient transfection of modified human embryonic kidney (HEK) cell lines. Here we show a case study of recombinant expression in HEK293 cells based on an ongoing research of extracellular domain of LLT1. Five cysteins contained within this lectin domain tend to cause misfolding and formation of aggregates. Using a multiple alignment tool with similar proteins from this family we identified a conserved region in primary sequence where LLT1 lacks its sixth cystein residue. Utilizing a site directed mutagenesis approach we were able to stabilize this structure by addition of this 'missing' residue. This led to significant improvement in yield and homogeneity of product that already enabled successful crystallization in two different crystalline forms and solution of the structure at 2.0 Å. Supported by Grant Agency (P207/10/1040, 303/09/0477 and 305/09/H008) and Ministry of Education (1M0505) of the Czech Republic, by Charles University (UNCE 204025/2012), and by EC funding through the SPINE2–COMPLEXES (031220) and P-CUBE (227764) projects.

P20-96
Atomic-level structure characterization of an ultrafast folding mini-protein denatured state

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Many tailed bacteriophages attach to their host cell via specialised fibre proteins. The fibre proteins of bacteriophages T4 and T7 are trimeric and contain an N-terminal phage attachment domain, a central shaft domain and a C-terminal receptor-binding domain. Here we present the structures of the C-terminal domains of the bacteriophage T4 long tail fibres and of the bacteriophage T7 fibre. The crystal structure of the receptor-binding tip of the bacteriophage T4 long tail fiber, which is highly homologous to the tip of the bacteriophage lambda side tail fibres, reveals an unusual elongated six-stranded anti-parallel beta-strand needle domain containing seven iron ions coordinated by histidine residues arranged co-linearly along the core of the biological unit. At the end of the tip the three chains intertwine forming a broader head domain, which contains the putative receptor interaction site. Amino acids 371–447 of the T7 fibre form a tapered pyramid with triangular cross-section composed of interlocked beta-sheets from each of the three chains. The triangular pyramid domain is connected at its narrow end to a carboxy-terminal three-blade beta-propeller tip domain, by three alpha-helices. The monomers of this tip domain each contain an eight-stranded beta-sandwich. The exact topology of the beta-sandwich fold is novel, but similar to that of knob domains of other viral fibres and the phage Sf6 needle. Several host range change mutants have been mapped to loops located on the top of this tip domain, suggesting the receptor-binding site may be located here, at the end of the fibre. The structures reveal previously unknown beta-structured fibrous folds, provide insights into the remarkable stability of the fibres and suggest a framework for mutations to expand or modulate receptor-binding specificity.

P20-97
The role of the molten globule state in the formation of amyloid-like structures by human alpha1-acid glycoprotein

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α_1-Acid glycoprotein (AGP) is a heavily glycosilated plasmatic protein belonging to the immunocalin family, a sub-group of the lipocalin family involved in immune response. The protein structure features a central β-barrel forming a hydrophobic binding site for a wide number of biologically-active exogenous and endogenous compounds, while the oligosaccharides allow for the interaction with, and recognition by, specific cell types of the immune system. Fourier-Transform infrared spectroscopy of temperature-induced denaturation of AGP revealed the presence of a molten globule (MG)-like folding intermediate at neutral and acidic pH values, with no such intermediate being detected in alkaline or strongly acidic environments. Interestingly, further heat denaturation of the MG state obtained at acidic pH values initiated aggregation of the polypeptide chains into intermolecular, anti-parallel β-sheets. This is accompanied by the typical

spectral changes occurring in the absorption and fluorescence emission spectra of the amyloid-binding dyes Congo Red and Thioflavin T, respectively, suggesting formation of fibrils featuring a cross-β architecture.

In order to probe in greater detail the connection of MG and the aggregation of AGP, time-resolved infrared spectra were collected with millisecond resolution after instant release of protons by the photoreactive 'caged' compound 1-(2-nitrophenyl)ethyl sulphate. A change in the time constant of antiparallel β-sheet formation could be measured, allowing us to assess the effect of temperature and pH on the MG-driven aggregation of AGP. The data suggest that partial or complete exposure of the aggregation-prone regions could trigger AGP assembly into amyloid structures.

P20-98
Effects on the thermal stability and enzymatic activity caused by substitutions of co-variant amino acids of the β-glucosidase from Spodoptera frugiperda

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The role of the active site residues of the β-glucosidase from *Spodoptera frugiperda* (SfβGlu) on the activity and substrate specificity was previously studied (Marana et al., 2001; Mendonça *et al.* 2008, Mendonça *et al.*, 2011). In the other hand, Statistical Coupling Analysis (SCA) revealed 23 co-variant positions in SfβGlu which are placed outside from the active site. Nevertheless, the presence of interaction networks among most (16) of these co-variant residues and the active site suggest that they could be important for SfβGlu activity and stability. We produced eight site-directed mutant SfβGlu containing replacements for alanine at the co-variant positions. Mutations W452A and W54A inactivated the enzyme, whereas the mutant F460A had only a residual activity. Moreover, mutations M57A, S445A and P203A changed the enzymatic activity when compared to the wild-type SfβGlu. Mutations N112A and K49A, which are distant from the active site, had also modulated the enzyme activity, probably via indirect contacts that affect the active site. Lastly, heat-inactivation of site-directed mutant SfβGlu demonstrated that the protein stability might be related to the degree of connection of the amino acids. Thus mutations of amino acids presenting few interactions had no effect on the enzyme stability, which exhibited a single exponential heat-inactivation at 45°C, while replacement of the highly connected amino acids produced a double exponential heat-inactivation, suggesting that enzyme denatures as two independent units.

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P20-99
NMR as a tool to target protein–protein and protein–ligand interactions

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Numerous biological processes depend on protein interaction with biological molecules. The challenge of targeting the binding modes between the protein partners is of fundamental importance in structure-based drug design. The identification of molecules with binding activity for the target protein usually requires the screening of much extended libraries of compounds. NMR plays an important role for the identification of ligands at the initial screening level and for subsequently guiding their optimization into drugs for therapeutic intervention. It also represents an extremely powerful tool for studying the formation and the structure of weak protein-protein complexes, which represent an interesting pharmaceutical target for drug discovery projects. We present here an approach that combines biotechnology and advanced NMR tools for studying the interactions of 'difficult' proteins involved in neurodegenerative and oncology diseases.

P20-100
Structure of the second pair of fibronectin type III repeats of the integrin β4 subunit

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The integrin α6β4 is a component of the hemidesmosomes (HD), protein complexes that mediate the stable anchoring of basal epithelial cells to the basement membrane [1]. The cytoplasmic domain of the β4 subunit is unique among the integrin family and it is responsible for most of the intracellular interactions of α6β4, including the interaction with other hemidesmosomal components. The cytoplasmic region of β4 contains four fibronectin type III domains (FnIII1 to FnIII4) arranged in two pairs separated by a region named the connecting segment. Upstream of the FnIII1 domain there is a Calx- β domain [2], while a 90-residue long C-terminal tail extends downstream of the FnIII4 domain. It is proposed that prior to HD assembly the cytoplasmic domain of β4 adopts a closed conformation stabilized by an intramolecular interaction between the connecting segment and the tail; binding to plectin would unleash β4 and favour the association of β4 with other components of the HD [3].

We have combined x-ray crystallography, small angle x-ray scattering (SAXS), mutagenesis, and biochemical analysis to characterize the second pair of FnIII domains of β4. The crystal structure of the FnIII3 was phased by molecular replacement and it was refined against data to 1.6 Å resolution. The crystal structure of the FnIII4 was phased by single isomorphous replacement with anomalous scattering using a mercurial derivative, and the structure was refined against native data extending to 1.8 Å resolution. The structure of the FnIII3-FnIII4 region was analyzed in solution by using SAXS. The FnIII3-FnIII4 tandem has a radius of gyration (Rg), calculated from the SAXS data, of 21 Å, which is significant smaller than the Rg of the FnIII1-FnIII2 (∼29 Å). We have modelled the low resolution structure of the FnIII3-FnIII4 region by using the SAXS data and *ab initio* methods. This region has a compact structure that consists apparently of two lobes. The limited resolution of the SAXS-based model hinders the unequivocal docking of the high resolution structures of the FnIII3 and the FnIII4 into the molecular envelope. Thus, we have used site direct spin labelling (SDSL) in combination with electron paramagnetic resonance (EPR) spectroscopy to obtain inter-domain distance restraints that will help us to elucidate the relative orientation of the FnIII3 and FnIII4 in solution. Our results have implications for the organization of the integrin β4 subunit and for its mechanisms of auto-inhibition and activation.

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P20-101
Monospecific MASP inhibitors identify MASP-1 as the activator of the lectin pathway and provide the first michaelis-complex structure of a MASP protease

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Lectin pathway is an antibody-independent activation route of the complement system. It provides immediate defense against pathogens and altered self-cells, but also causes severe tissue damage after stroke, heart attack and other ischemia reperfusion injuries. The pathway is triggered by target-binding of pattern recognition molecules leading to activation of associated zymogen MASPs (mannose-binding lectin-associated serine proteases). Until now the autoactivating MASP-2 has been considered as the autonomous initiator of the proteolytic cascade. The role of the much more abundant MASP-1 protease was controversial. We evolved unique, monospecific inhibitors against MASP-1 and MASP-2 by phage display. These inhibitors were used as unique reagents to reveal a completely novel mechanism of lectin pathway activation. In normal human serum MASP-2 activation strictly depends on MASP-1. MASP-1 activates MASP-2 and moreover, inhibition of MASP-1 prevents autoactivation of MASP-2. Furthermore we demonstrated that MASP-1 produces 60% of C2a responsible for C3 convertase formation. To understand structural basis of the highly selective interaction between MASP-2 and its physiological substrates we provide a complex structure of MASP-2 and our novel substrate-like inhibitor at a resolution of 1.28 Å. It reveals significant structural plasticity for MASP-2 suggesting that induced fit should contribute to the extreme specificity of the enzyme.

P20-102
Molecular evolution of the CYTH superfamily of proteins

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The CYTH superfamily of proteins was named after the two founding members, the CYaB adenylyl cyclase from *Aeromonas hydrophila* and the human 25-kDa THiamine triphosphatase (ThTPase). Members of this superfamily of proteins exist in all organisms including bacteria, archaea, plants and animals (except in birds) and can be traced back to the Last Universal Common Ancestor. They are characterized by a consensus sequence including several charged residues involved in divalent cation and triphosphate binding. Indeed, all members of the CYTH family that are characterized act on triphosphate derivatives and require at least one divalent cation for catalysis. The *Nitrosomonas europaea* (1) and *E. coli* CYTH proteins are specific inorganic triphosphatases. We propose that inorganic triphosphate (PPP_i), the most simple triphosphate compound that can be imagined, is the primitive substrate of CYTH proteins. Other enzyme activities such as adenylate cyclase (in *A. hydrophila*), mRNA triphosphatase (in fungi and protozoans) and ThTPase (in metazoans) activities are secondary acquisitions. We show that ThTPase activity is not limited to mammals, but Sea anemone and Zebrafish CYTH proteins are already specific ThTPases and the acquisition of this enzyme activity is linked to the presence of a Trp (W53 in mammalian ThTPases) residue involved in the binding of the thiazole heterocycle of the thiamine molecule. The importance of W53 for the specificity of mammalian ThTPases is confirmed by site-directed mutagenesis. Furthermore, we propose a conserved catalytic mechanism between inorganic triphosphatases and ThTPases, based on a catalytic dyad comprising a Lys and a Tyr residue, explaining the alkaline pH optimum of CYTH proteins.

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P20-103
Biotransformation of aromatic amines in contaminated soils: characterization of fungal acetyltransferases

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Aromatic amines (AA) represent one of the most important classes of environmental pollutants. Living species use several xenobiotic metabolic pathways to protect themselves against the toxic effects of these pollutants. Arylamine *N*-acetyltransferase (NAT) are xenobiotic-metabolizing enzymes (XME) which catalyze the transfer of an acetyl group from acetyl-coA (AcCoA) to AA and their *N*-hydroxylated metabolites. The *N*-acetylation of these chemicals has been shown to detoxify them. The study of these mechanisms may pave the way for novel bioremediation applications. Interestingly several *Trichoderma* species possess NAT genes. *Trichoderma* spp. are abundant soil borne ascomycetes, successful colonizers and efficiently competitors. The aim of this work was (i) to characterize NAT enzymes of *Trichoderma virens* (TvirNAT) and *Trichoderma reesei* (TreeNAT), (ii) to study the fungal tolerance to AA, and (iii) to investigate their capacity to biotransform AA to acetyl AA. NAT activity was measured with different aromatic substrates (drugs, pesticide residues and industrial chemical products). TvirNAT and TreeNAT showed the same substrate specificity and very similar catalytic efficiencies. However TvirNAT efficiency was 0–7 fold higher for most of substrates tested here, compared to TreeNAT. Tolerance assays showed the capacity of these strains to resist and grow in the presence of high concentrations of AA, especially *T. virens*. Moreover, both strains have the capacity to transform AA present in the medium by *N*-acetylation pathway. These findings expand the understanding of the role of xenobiotic-metabolizing enzyme and in particular of NATs in the adaptation of fungi to their chemical environment and provide a basis for new systems for the bioremediation of contaminated soils.

P20-104
Interaction of PARP2 with DNA structures mimicking DNA repair intermediates and comparative analysis of PARP1/PARP2 influence on BER proteins

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Poly(ADP-ribosyl)ation is a posttranslational protein modification significant for genomic stability and cell survival in response to DNA damage. Poly(ADP-ribose)polymerases (PARPs), which

catalyze poly(ADP-ribosyl)ation, constitute a large family of 17 proteins, but only PARP1 and PARP2 can be immediately activated by DNA damage. Whereas the role of PARP1 in response to DNA damage has been widely illustrated, the contribution of PARP2 has not been studied in detail. To find out specific DNA targets we evaluated affinity of PARP2 to several DNAs mimicking intermediates of different DNA metabolizing processes, and tested these DNA as 'activators' of PARP1 and PARP2. The influence of PARP2 (in comparison with PARP1) on several base excision repair (BER) proteins (Polβ and FEN1) has been investigated. As a whole, both PARPs negatively regulate the activity of the BER enzymes. The FEN1 influence on PARPs is differential: the PARP1 activity is inhibited while the PARP2 one is enhanced. Interplay between PARP1, PARP2, XRCC1 and polβ was also studied. The capability of PARP2 to interact with a key DNA intermediate of BER, abasic sites has been demonstrated for the first time. Therefore, our results testify to the complicated multilevel regulation of DNA synthesis during BER pathways under coordinated action of PARP1 and PARP2.This work was partially supported by RFBR projects 10-04-01083, 11-04-00559.

P20-105
How to determine the size of nuclei of protofibrils from the concentration dependence of lag-time duration of amyloid formation?

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The question about the size of nuclei of protofibrils formed by different proteins and peptides is yet open. By the nucleation mechanism, the formation of protofibrils begins from the thermodynamic unfavorable steps resulting in the formation of a critical nucleus consisting of n monomers. The kinetic model of the process of formation of amyloid fibrils is suggested in our work allowing us to calculate the size of the nucleus using kinetic data. In addition to the stage of nucleation, the given model includes both a linear growth of protofibrils (proceeding only at the cost of attaching of monomers to the ends) and an exponential growth of protofibrils at the cost of branching and fragmentation. Theoretically, only the exponential growth is compatible with the existence of a lag-period in the fibril formation kinetics. The obtained analytical solution and computer modeling allow us to determine the size of the nucleus from the experimentally obtained concentration dependences of the relationship between the lag-time duration and the time of growth of amyloid fibrils. In the case of insulin this relationship does not depend on the protein concentration. According to the elaborated theory it means that the size of the nucleus corresponds to that of the monomer. This study was supported in part by the Russian Foundation for Basic Research (grant 11-04-00763), Russian Academy of Sciences (programs ''Molecular and Cell Biology'' (01200959110 and 01200959111) and ''Fundamental Sciences to Medicine'').

P20-106
Functional and structural interactions of Nb, V, Mo and W oxometalates with the sarcoplasmic reticulum $Ca2^{+}$ -ATPase reveal new insights into inhibition processes: a combination of NMR, Raman, AA and EPR spectroscopie with kinetic studies

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Oxometalates, in particular decavanadate, is a potent inhibitor (IC_{50} 15 μM) of the hydrolytic activity of sarcoplasmic reticulum Ca^{2+}-ATPase, a transmembrane protein involved in calcium translocation and responsible for muscle contraction relaxation [1]. Here, we further investigate the interaction of niobate, vanadate, molybdate and tungstate oxometalates with the sarcoplasmic reticulum Ca^{2+}-ATPase in order to reveal new insights into the metal inhibition mechanisms, by combining NMR, AAS, Raman and EPR spectroscopie with kinetic studies. The decavanadate calcium pump interaction is promoted by ATP, and prevented by the iso-structural and iso-electronic decaniobate, as observed by NMR spectroscopy. On the other hand, atomic absorption spectroscopy (AAS), indicatest that decavanadate (V_{10}) binds in the same extent to all protein conformations occurring during the process of calcium translocation, namely E1, E1P, E2 and E2P. Decavanadate ATPase activity inhibition is shown to be competitive at lower concentrations (10 μM) and non-competitive for higher concentrations (50 μM), whereas decaniobate shows a non-competitive inhibition at all concentrations. Although only vanadate and decavanadate induces protein cystein oxidation, gluthatione – a known intracelullar antioxidant – does not revert the inhibition promoted by any of the oxometalates analysed. Finally, the Raman studies suggested that decavanadate, decaniobate and vanadate induce similar Ca^{2+}-ATPase conformational changes – different from E1 or E2 – that differ from those observed upon molybdate and tungstate interaction. In conclusion, decavanadate shows a specific interaction with the calcium pump, as opposed to the other oxometalates, and induces cysteine oxidation and multiple inhibition types. The ATPase inhibition is not reverted by antioxidants and shows a specific mode of interaction between the decavanadate and the Ca^{2+}-ATPase which is not affected by any of the protein conformations that occurs during the process of calcium translocation.
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P20-107
Conformational studies of human leukocyte antigens: when infrared spectroscopy meets immunology

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Human leukocyte antigen (HLA) class I molecules consist of a polymorphic heavy chain (HC), non-covalently associated β_2-microglobulin (β_2m), and a peptide. Certain HLA-B27 subtypes,

such as B*2704 and B*2705, are associated with the autoimmune disease ankylosing spondylitis (AS), whereas others, like B*2706 and B*2709, are not [1]. The molecular basis for the association of HLA-B27 with AS is not understood.

HLA-B27 complexes loaded with different peptides were studied by isotope-edited IR spectroscopy. For this purpose, the membrane-distal segments of the different HC's were recombinantly expressed and reconstituted together with the corresponding peptides and uniformly ^{13}C-labeled β_2m. Our analyses [2-5] revealed the existence of previously undetected subtype-specific conformational and dynamic differences between HLA-B27, which could lead to distinct interactions with various ligands.

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P20-108
Life and death of proteins destined to the mitochondrial intermembrane space

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Nearly all mitochondrial proteins are synthesized in the cytosol and translocated into the organelle. Many of them have signal presequences that are proteolytically removed by specific peptidases upon their selective import into one of mitochondrial compartments: the matrix, the inner membrane or the intermembrane space (IMS). Additionally various types of non-cleavable targeting and sorting signals exist. The diversity of signals is matched by the specific import pathways. Many IMS proteins share a coiled coil-helix-coiled coil-helix (CHCH) domain, stabilized by disulfide bonds formed within the conserved cysteine motifs. Upon protein synthesis and release to the cytoplasm, the cysteine residues of the IMS proteins stay reduced. The IMS proteins are transported across the outer mitochondrial membrane via the TOM complex (translocase of the outer membrane), a main entry gate for all mitochondrial precursor proteins. The protein precursors destined to the IMS are recognized by the mitochondrial import and assembly machinery MIA. Subsequently, the oxidative folding catalyzed by MIA is required to trap them in the IMS. We aim to understand the fate of IMS proteins under the conditions of oxidative folding restrictions. Under these conditions, for example in the mutants of the essential MIA components, Mia40 and Erv1, the IMS proteins accumulate neither in mitochondria nor in the cytosol, suggesting their efficient degradation. We present the results of systematic analyses undertaken by us to determine degradation pathways that are involved in cellular homeostasis of the IMS proteins.

P20-109
Temperature dependence of the activity alterations induced by acriflavine in horseradish peroxidase

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Acriflavine is known to interact with DNA and, in recent studies, we showed that acriflavine also interacts with proteins. Here, the effect of temperature on interaction between acriflavine and horseradish peroxidase C (HRPC) was investigated.

HRPC was preincubated at 25, 35 and 45°C with various acriflavine concentrations for up to 60 min. The enzymatic activity was then assayed at room temperature, under steady-state kinetics conditions, by monitoring the H_2O_2-mediated oxidation of o-dianisidine at 460 nm, in citrate buffer 0.1 M, pH 4. Preincubation at 25 and 35°C led to activity stimulation with up to 0.6 mM acriflavine, and to activity inhibition with higher concentrations. Preincubation at 45°C led to activity inhibition, regardless of acriflavine concentration. Inhibition extent depended on temperature, duration of preincubation and acriflavine concentration; enzymatic activity was reduced by 50% after preincubation at 25, 35, 45°C with, respectively, 0.71, 0.70, 28 mM acriflavine for 30 min, or 0.43, 0.23 and 0.14 mM acriflavine for 60 min. With o-dianisidine as varied substrate, acriflavine was noncompetitive inhibitor at lower concentrations and mixed inhibitor at higher concentrations; change in inhibition type occurred more rapidly as temperature and duration of incubation increased. With H_2O_2 as varied substrate, acriflavine was noncompetitive inhibitor at 25°C and either noncompetitive or mixed inhibitor at 35 and 45°C. Acriflavine caused an electrophoretic mobility shift of HRPC in 1% agarose gel, indicating formation of a complex with the protein. Data showed that acriflavine bound to HRPC, altered its activity and affected differentially the reducing substrate- and peroxide-binding sites; alterations were enhanced at higher temperatures.

P20-110
Vicinal thiols are involved in adhesion of endothelial cells

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Activation of integrins, transmembrane receptors that mediate cell adhesion and migration, is accompanied by a series of conformational rearrangements resulting in changes in affinity and avidity. Several observations indicate that conformational changes induced by ligand interaction with integrins lead to exchange of disulfide bonds within the integrin molecule, which stabilizes the altered conformation. Closely spaced thiols in proteins that interconvert between the dithiol form and disulfide bonds are called vicinal thiols. The purpose for this study was to examine whether vicinal thiols are involved in adhesion process of endothelial cells. The manganese ions are know to affect the thiol-disulfide balance and activate integrin to maximal affinity. In the present study, we attempt to explain whether activation of integrins in endothelial cells by Mn^{+2} might involve vicinal thiols.

Human umbilical vein endothelial cells (HUVEC) were cultured in medium 200 supplemented with low-sereum growth

supplement. Labeling of sulfhydryl groups was performed using the poorly membrane-permeable maleimide reagent (MPB). Protein labeled with MPB were precipitated using avidin-Sepharose, electrophoresed and transferred on nitrocellulose, Biotinylated proteins were detected using streptavidin-horseradish peroxidase with chemiluminescent substrate. In some experiments, the MPB-labeled cells were used for immunoprecipitation performed with monoclonal or polyclonal antibody to αvβ3 integrin. For cell adhesion assay the plates were coated with fibrinogen, fibronectin or vitronectin. In some cases the cell were pretreated with phenylarsine oxide (PAO) and added to the plate. The binding of vitronectin or LM609 antibody to endothelial cells activated by manganese ion were evaluated by flow cytometry method.

The studies with membrane-impermeable reagent 3-N-maleimidylpropionyl biotin (MPB) demonstrate that exposure of endothelial cells to Mn^{+2} results in the appearance of surface protein thiol groups, which can found in αvβ3 integrin. Phenylarsine (PAO), a reagent that binds vicinal thiols inhibit adhesion of endothelial cells activated by Mn^{+2}. Flow cytometry experiments showed that PAO abolish the binding of vitronectin and LM609 antibody to endothelial cells activated by Mn^{+2}. Additionally, PAO inhibit sulfhydryl labeling of thiols in αvβ3 molecule.

The αvβ3 contains vicinal thiols that provide sites for redox regulation of function of this integrin. Vicinal thiols are involved in activation of αvβ3 integrin during adhesion of endothelial cells.

P20-111
Autoacetylation activity in the PB2 subunit of RNA-dependent RNA polymerase of influenza A virus

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The RNA-dependent RNA polymerase of the influenza A virus, which is constituted by PA, PB1, and PB2 subunits, is essential for the viral transcription and replication. Above all, the PB2 subunit is known to bind cap structures of host mRNAs for initiating transcriptional activity of viral mRNAs. To investigate the new functions of the PB2, we searched proteins containing similar tertiary structure with the PB2, and found that the structure of the middle part of the PB2, which contains the cap-binding domain, was significantly similar to that of bacterium-derived acetyltransferase, suggesting that the PB2 subunit harbors acetyltransferase activity. Then, the recombinant protein of the PB2 cap-binding domain (322th to 484th amino acid residues) was expressed in *Escherichia coli* and purified. Interestingly, incubation of this partial PB2 and radioisotope-marked acetyl coenzyme A (acetyl-CoA) showed that the cap-binding domain of the PB2 had autoacetyltransferase activity. Lineweaver-Burk plot estimated that the Km value of the domain for acetyl-CoA was around 0.65 μM. Autoacetylation level of the PB2 was dependent on the concentration of acetyl-CoA and duration of incubation. This autoacetyltransferase activity was significantly blocked by CoA and HAT (histone acetyltransferase) inhibitors, such as epigallocatechin-3-gallate, anacardic acid and garcinol, those could block kinds of HATs (Tip60, PCAF, p300/CBP and GCN5) activities. This is the first report showing that the RNA polymerase of influenza A harbors acetyltransferase activity.

P20-112
Shedding light on the most C-terminal RNA binding motif of HuR: its role in RNA recognition

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HuR is a 32-kDa multidomain protein constituted by three RNA Recognition Motifs (RRMs), with a canonical topology $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_4$, ubiquitously expressed in vertebrates. HuR stabilizes Adenosine-Uridine Rich Elements (AREs) at 3′UTR regions of the mRNAs, contributing to cell cycle, proliferation, immune activation, stress response and carcinogenesis in the cell[1]. The protein is well-characterized *in vivo*, but little information is available at the biophysical level, except for the crystal structure of RRM1[2] and the thermal stability of the two N-terminal RRM1-RRM2 adduct[3].

The most C-terminal RRM (RRM3) is the great unknown domain due to its solubility problems in the context of HuR full length (HuR-FL). We have developed a strategy to isolate RRM3 for structural analysis of the domain. Indeed, RRM3 is a suitable structural model, since it is independent of the RRM1-RRM2 two-domain-construct, as inferred from NMR measurements and thermal stability studies. Based on NMR experimental data, structural model of RRM3 wild-type (RRM3-WT) was created by the CS23D server[4] to compare it with the phosphomimetic mutant RRM3-S318D. This residue is known to be phosphorylated *in vivo* by the PKCδ kinase, increasing the RNA binding of HuR FL[5], regardless of whether RRM3 makes direct contact to RNA. Our NMR and CD titrations of both RRM3-WT and RRM3-S318D with 5-mer RNA molecule (′5-UUUUU-3′) reveal that not only those RRM3 residues at the β-sheet are involved in the complex formation, but also their dissociation affinity constants differ for RRM3-WT and RRM3-S318D.

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P20-113
Comparative biochemical analysis of the major yolk protein in the sea urchin egg and coelomic fluid

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The sea urchin major yolk protein (MYP) has been attributed a vitellogenin-like role as a source of nutrients for the developing embryo and larva. However, we now know that the sea urchin MYP has no sequence homology to any knowm vitellogenins and, in contrast to the vitellogenins, is neither gender specific nor exclusively localized to the female gonad. The MYP is present in both the testis and ovary as well as the coelomic fluid of the adult. Additionally, we and others have shown that the egg MYP can facilitate membrane-membrane interactions in a Ca^{2+}-dependent manner. Much less is known about the biochemical properties and activities of the coelomic fluid (CF)-localized MYP. We have therefore begun a comparative biochemical analysis of the egg and coelomic fluid MYPs. We have analysed fractions, enriched in either the egg or CF MYPs, by SDS-PAGE in the presence or absence of reducing agent. Under reducing conditions, two species were seen in each fraction, with the CF MYP species migrating with slightly higher apparent mol masses than the corresponding egg species: 250- and 180 kDa compared to 240- and 170 kDa, respectively. In the absence of reducing agent, a single species was seen in each fraction at 250 kDa (CF) or 240 kDa (Egg). Two-dimensional gel electrophoresis confirmed that the 180- and 170 kDa species comigrated with the higher mol mass species in the absence of reducing agent. We next utilized V8 protease peptide mapping to investigate the primary structural relationship between the four species. Our results clearly show that all four polypeptides have identical primary structures. We further analysed the four species by sucrose density gradient ultracentrifugation (15–30%). Each of the four species exhibited a unique elution profile. This latter result suggested that each polypeptide was unique in terms of its size and/or shape indicating differences in the tertiary and/or quaternary structures of these species. Collectively, these results identify structural differences between the egg and coelomic fluid MYPs which may result in differing functional capabilities between these species.

P20-114
Hydrogenases: highly active and selective biological catalysts to inspire Pt-free hydrogen energy technology

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Hydrogen is often described as a 'fuel for the future' but microbes have been using H_2 as an energy source for billions of years. Hydrogenases are the metalloenzymes which are produced by microbes to catalyse either H_2 oxidation or H_2 production. Hydrogen-activating catalysts like Pt often also react with O_2 and CO, so most manmade H_2-energy technologies require ultrapure gases. However, certain bacteria like *E. coli* and *Salmonella* produce [NiFe]-hydrogenases which are highly selective and can operate in air, and are extremely tolerant to CO. To understand the structural control centres of this highly active and efficient biological catalysis we use electrochemistry, EPR, crystallography and molecular biology. Such work enhances our understanding of how different hydrogenases enable bacteria to maximally optimise the use of hydrogen as a key metabolite. It also aids our understanding of the key features which are needed to engineer rare-metal free catalysts.[1,2]

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P20-115
Characterization of the subunits of the giant extracellular hemoglobin of *Rhinodrilus alatus* (HbRa) by MALDI-TOF-MS: evidence for strong interaction with cationic surfactants DTAB and CTAC

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Giant extracellular hemoglobin of *Rhinodrilus alatus* (HbRa) has a molecular mass (*MM*) very similar to that of *Glossoscolex paulistus* (HbGp), of 3.6 MDa (see Tabak M. et al. chapter 15 in the book 'Stoichiometry and Research-The importance of quantity in Biomedicine', Intech Open Science, 2012). In this work, further studies are performed by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) of the *MM* masses of HbRa subunits as well as the effects of two cationic surfactants, dodecyl trimethyl ammonium bromide (DTAB) and cethyl trimethyl ammonium chloride (CTAC). The mass spectrum of the whole oligomer shows an intense monomeric peak *d* at 16.18 kDa, a peak corresponding to the trimer *abc* at 51.40 kDa and peaks in the range 25–32 kDa corresponding to linkers. Beta-mercaptoethanol reduces the disulfide-bonded trimer and several peaks appear in the range from 16 to 18 kDa. Besides the monomeric peak *d* at 16.18 kDa, three intense peaks at 16.70, 17.15 and 17.31 kDa are observed, associated to the monomeric subunits *a*, *b* and *c*. Both cationic surfactants, DTAB and CTAC, induce a significant effect: at least seven new peaks are observed besides the main monomeric one, *d* at 16.18 kDa, with *MM* corresponding to the addition to the monomer of multiple values of the masses of one DTA^+ or CTA^+ cation. In the same way as occurs for the HbGp-CTAC system (Oliveira et al. Int. J. Biol. Macromol. 42, 111–119, 2008), HbRa binds strongly up to seven cationic surfactant molecules to its monomeric subunit. Overall, our results suggest a very similar oligomeric structure for HbRa, similar to other giant extracellular hemoglobins.

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P20-116
Specificity of DNA recognition by the zinc finger transcription factor YY1

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Yin Yang 1 (YY1) is a ubiquitous, multifunctional human transcription factor that takes part in the regulation of various processes such as carcinogenesis, B-cell development or some viral infections. Here, we have used fluorescence anisotropy and SPR to study the interaction of recombinant YY1 with selected DNA sequences and have found that YY1 binds to its specific sites with relatively low affinity ranging from high nanomolar to low micromolar. Further experiments allowed us to determine the affinity towards nonspecific DNA to be between 30 and 40 μM and the calculated specificity ratios are low, between 3 and 220. Additional kinetic measurements for the YY1-DNA interaction revealed fast on- and off-rates showing a dynamic interaction that is regulated by changes in both ka and kd. Based on these findings, we propose that YY1 requires an association with additional co-factors to bind to DNA and regulate transcription.

P20-117
Quantitation of fibroblast activation protein (FAP)-specific protease activity in mouse and primate fluids and organs including cirrhotic liver

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FAP is a member of the prolyl oligopeptidase family of proteases which specialises in cleaving the post proline bond. This family also includes dipeptidyl peptidase 4 (DPP4). FAP has both dipeptidyl- and endopeptidyl peptidase activities and can thus cleave all substrates used to identify the other members of this enzyme family. This has made specific FAP detection difficult up to now. Recently, however, a FAP-specific substrate (ARI-3144) has been developed and used to study FAP expression in tomours (Poplawski *et al*, 2012 Manuscript in preparation).

This study confirmed the *in vivo* specificity of this novel substrate and specifically quantified FAP enzyme activity in tissue and blood samples from human, baboon and mouse. Quantification used an amino-4-methylcourmarin (AMC) standard curve.

FAP was detected in all wild type mouse organs but mostly at very low levels. In contrast, all organs and blood from FAP gene knockout mice were negative, thus confirming, in all organs, the *in vivo* specificity of this substrate for FAP throughout the body. The highest levels of FAP in mice were found in uterus, submaxillary gland, lymph node, ovary, skin, adrenal, bone marrow and skeletal muscle showing a range of 1.5–6 pmol AMC/min/mg wet weight tissue. The large vital organs such as heart, liver, lung and kidney had little FAP (<0.3 pmol AMC/min/mg wet weight tissue). The organ distribution in baboon was similar to mouse. High-FAP organs in baboon included skin, ovary, epididymis, bladder, adipose tissue, nerve and colon (16–45 pmol AMC/min/mg total protein). DPP4 enzyme levels were also measured in baboon organs, demonstrating a different expression pattern, as expected.

FAP was detected at about 330 pmol AMC/min/ml in baboon plasma, which was ~20 fold less than mouse plasma and ~1.3-fold less than human plasma. Other baboon bodily fluids, such as urine and bile, had no detectable FAP enzyme activity. Plasma FAP levels were similar in both wildtype and DPP4 gene knockout mice.

Finally, FAP was examined in some diseased states with increased activity in baboon tumours and diseased skin and lymph nodes compared to normal controls. Furthermore, there was a ~15-fold increase in FAP enzyme activity in cirrhotic compared to non-diseased human liver, which was confirmed by Western blotting.

The mouse and baboon data are comparable to a previous study of mouse and monkey. Thus, this new reagent is a useful and robust tool for the specific measurement of both soluble and cell-surface FAP enzyme activity.

P20-118
Molecular modeling suggests a novel role for ikaros ZF4 in DNA and protein interactions

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The IKAROS gene is alternatively spliced to produce multiple zinc finger proteins. The most abundantly expressed Ikaros splice forms are DNA binding proteins that are essential for hematopoietic differentiation and exert tumor suppressor activity in leukemia. The specific mechanisms by which Ikaros exerts it tumor suppressor activities are unknown. The Ikaros protein is known to both activate and repress target gene expression and to participate in chromatin remodeling through interactions with the SWI/SNF nucleosome remodeling complex and histone deacetylase (HDAC)-containing complexes. Full length Ikaros (IK1) has six zinc fingers grouped as 4 tandem zinc fingers in the N-terminal DNA binding domain (ZF1-ZF4) and the tandem ZF5-ZF6 in the C-terminal protein interaction domain present in all Ikaros isoforms. IK1 and IKX (lacks ZF4) are the predominant isoforms in normal hematopoietic cells. While IK1 plays a critical role in lymphoid differentiation, expression of IKX has been linked to cells with myeloid differentiation potential. We have modeled the ZF1-ZF4 module with DNA target sequences associated with various Ikaros functions including the MYC and BS4 target DNA sequences. We conclude that only three of ZF1-ZF4 are likely to bind in the major groove of the target sequence and that ZF4 may assist ZF5 and ZF6 in promoting Ikaros dimerization and/or play a role in Ikaros interactions with other proteins. Such a role for ZF4 could provide a molecular basis for potential differences in the roles of IK1 and IKX in lymphoid versus myeloid differentiation.

P20-119
A new approach for treatment of type 2 diabetes: Sitagliptin

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Type 2 diabetes mellitus is the most common form of diabetes and characterized by reduced insulin secretion and hyperglycemia. Pancreatic islet cell dysfunction in type 2 diabetes involves both defective insulin secretion from beta cells and increased

glucagon secretion from alpha cells. Somatostatin inhibits insulin and glucagon secretion in the pancreas. The long term treatment studies with dipeptidyl- peptidase-4 enzyme (DPP-4) inhibitors in diabetic animals showed that islet cell function protects and increased beta cells survives. Sitagliptin, a novel DPP-4 inhibitor, is a new therapeatic agent for type 2 diabetes. In this study, we aimed to investigate the effect of sitagliptin in the main cells of newborn STZ-diabetic rat pancreas using insulin, glucagon and somatostatin antibodies by immunohistochemistry. Wistar albino newborn rats divided into four groups. Group I: The saline was administrated intraperitoneally (i.p) to rats. Groups II: Newborn rat group, from the day five sitagliptin that dissolved in the saline injected 1.5 mg/kg subcutaneous (s.c) for 15 days. Group III: Second day after the birth, 100 mg/kg streptozotocin (STZ) administrated i.p a single dose to the newborn rats. Group IV: Diabetic animals given sitagliptin for 15 days (STZ + Sit). Pancreas samples were fixed in neutral saline and processed routine paraffin embedding for microscopic investigation. Pancreas sections were stained by insulin, glucagon and somatostatin antibodies. Insulin immune positive cells increased in STZ + Sit as compared to the STZ-diabetic rats insignificantly. Glucagon immune positive cells increased in STZ + Sit group as compared to the diabetic group significantly ($p < 0.001$). There was no significant difference for somatostatin immune positive cells for all groups. The result showed that sitagliptin treatment has a protective effect to some extent on the diabetic rats. However, further investigation is necessary for sitagliptin in the treatment of diabetes mellitus.

P20r-120
Crystal structures of the receptor-binding C-terminal domain of bacteriophage T4 and T7 tail fibres

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Many tailed bacteriophages attach to their host cell via specialised fibre proteins. The fibre proteins of bacteriophages T4 and T7 are trimeric and contain an N-terminal phage attachment domain, a central shaft domain and a C-terminal receptor-binding domain. Here we present the structures of the C-terminal domains of the bacteriophage T4 long tail fibres and of the bacteriophage T7 fibre. The crystal structure of the receptor-binding tip of the bacteriophage T4 long tail fiber, which is highly homologous to the tip of the bacteriophage lambda side tail fibres, reveals an unusual elongated six-stranded anti-parallel beta-strand needle domain containing seven iron ions coordinated by histidine residues arranged co-linearly along the core of the biological unit. At the end of the tip the three chains intertwine forming a broader head domain, which contains the putative receptor interaction site. Amino acids 371-447 of the T7 fibre form a tapered pyramid with triangular cross-section composed of interlocked beta-sheets from each of the three chains. The triangular pyramid domain is connected at its narrow end to a carboxy-terminal three-blade beta-propeller tip domain, by three alpha-helices. The monomers of this tip domain each contain an eight-stranded beta-sandwich. The exact topology of the beta-sandwich fold is novel, but similar to that of knob domains of other viral fibres and the phage Sf6 needle. Several host range change mutants have been mapped to loops located on the top of this tip domain, suggesting the receptor-binding site may be located here, at the end of the fibre. The structures reveal previously unknown beta-structured fibrous folds, provide insights into the remarkable stability of the fibres and suggest a framework for mutations to expand or modulate receptor-binding specificity.

P20-121
Molecular insights into the electron transfer mechanism in the *Rhodobacter capsulatus* FPR

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Ferredoxin:NADP(H) reductases (FNRs/FPRs, EC 1.18.1.2) carry out the transference of two electrons between two molecules of a mono-electron carrier (flavodoxin or ferredoxin) and the NADP(H), by a reversible mechanism1. FPR from *R. capsulatus* is a bacterial ferredoxin:NADP(H) reductase proposed to shuttle electrons from the NADPH pool of the cell to the flavodoxin NifF, a potential electron carrier of the nitrogenase2,3. The FPR and NifF structures have been recently resolved and, in order to elucidate the electron transfer mechanism of this redox system, we are going to undertake the structural characterization of the several active-site mutants of the FPR enzyme by X-Ray Crystallography.Crystallization assays will be carrying out using the recombinant proteins and their structures will be solved by the Molecular Replacement Method on the basis of the native FPR structure (PDB code 2BGI).

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P20-122
Crystal structure of FAD-containing ferredoxin-NADP+ reductase from *Xanthomonas axonopodis* pv. citri

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Ferredoxin-NADP(H) reductases (FNRs, EC 1.18.1.2) constitute a family of hydrophilic, FAD-containing monomeric enzymes that deliver NADPH or low potential one-electron donors to redox-based metabolisms in plastids, mitochondria and bacteria. In heterotrophic bacteria, the FNR activity provides reduced ferredoxin and flavodoxin to diverse reactions. Based on phyloge-

netic analysis the prokaryotes FNR variants present (collectively known as FPRs) have been classified into subclass I and II represented by the *Azotobacter vinelandii* and the *Escherichia coli*, respectively. FPR prototypes. Structures of bacterial and plastidic FNRs contain two distinct domains; the C-terminal domain that has a binding site for NADP(H) and the N-terminal region that binds the cofactor FAD. In bacterial enzymes, the FAD is in a bent conformation that could be the explanation for the very low turnover rates for NADPH oxidation exhibited in the diaphorase activity of the bacterial FPRs with respect to those of plastidic enzymes. Additionally, a subdivision of subclass I bacterial FNRs, IA and IB, was proposed based on differences of their carboxy-terminal primary sequences. To better understand the structural and functional divergence between subclass I FPRs, in the present work we have determined the crystal structure of *Xac*FPR to a resolution of 1.5 Å. This FPR comes from the parasite *Xanthomonas axopodis* pv. citri, a Gram-negative bacterium responsible for citrus canker, a severe disease that affects most commercial citrus cultivars. The final structure reveals that *Xac*FPR adopts many structural characteristics of the bacterial subclass IA, although some structural differences among FPRs from subclass IA and IB have been detected. The ones centered at the FAD environment and at the $NADP^+$ binding site could help to better understand the kinetic behavior of these oxidoreductases. Furthermore this enzyme represents a potential target to treat infections caused by *Xanthomonas axonopodis* pv. citri The information obtained may provide a rationale to develop new inhibitors of these enzymes.

P20-123
Structure of the Rbg1-Tma46 complex reveals new functional domains and their role in polysome recuitment

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Developmentally Regulated GTP binding protein (DRG) is an uncharacterized member of the TRAnslation FACtor (TRAFAC) class of GTPases, thought to play, along with their binding partners DRG Family Regulatory Protein (DFRP), an important role in cell growth and differentiation. Highly conserved in all and only eukaryotic organisms, there are two DRG genes, *drg1* and *drg2*, whose structure and function is largely unknown. A direct involvement in translation was earlier observed when the yeast Drg1 homologue, named as Rbg1 for Ribosome Binding GTPase, in complex with Tma46, the Translation Machinery Associated protein 46 (yeast Dfrp1 homologue) associated with translating ribosomes. It was also demonstrated that a triple deletion mutant lacking *drg1*, *drg2* alongwith*slh1* (a putative RNA helicase) results in a negative growth phenotype in yeast (Daugeron M.C., 2011). We recently obtained the first crystal structure of Rbg1 in complex with the C-terminal domain of Tma46 to 2.67 Å resolution (unpublished data). Rbg1 was found to contain the well-known GTP binding domain typical to GTPases and the functionally unknown C-terminal TGS domain. Surprisingly, a hereto-unknown domain containing two subdomains, one, a helix-turn-helix and the other, with an unusual βαβ fold with similarity to the C-terminal domain of the Ribosomal S5 protein, was also discovered. Tma46 C-terminal fragment was seen to be highly unstructured wrapping around Rbg1. The structure of the Rbg1-Tma46 complex opened up interesting questions on the importance of its individual components on cell growth and function. To this end, several domain deletion mutants were designed which gave insights on their role on yeast cell growth, polysome association and the GTPase activity of Rbg1.

P20-124
The intracellular localization of regulatory and capsid proteins of the densovirus of German cockroach, Blattella germanica

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The densovirus of German cockroach, Blattella germanica (BgDNV), belongs to a group of single stranded DNA viruses infecting arthropods. It is characterized by 20 nm icosaedral capsid and 5335 nt genome. BgDNV possesses three different capsid proteins (VP1-3) and three regulatory proteins: NS1, the main parvoviral regulatory protein, and NS2 and NS3 whose functions are completely unknown.

The objective of the current research was to determine the intracellular localization of BgDNV proteins that could further shed light on BgDNV protein functions and regulation of the viral life cycle. Preliminary bioinformatic analysis revealed the presence of NLS sequences in NS1, NS2, and all three VP proteins and NES sequences in NS1 and VP2 proteins.

Immunofluorescent analysis of BgDNV-infected BGE-2 cells with corresponding monoclonal antibodies demonstrated nuclear localization of NS1 and VP1 proteins. Subsequent analysis of intranuclear localization using confocal microscopy showed unusual perinuclear localization of NS1, never described before for DNVs.

As NS2 and NS3 monoclonal antibodies were not suitable for use with IFA to unravel the intracellular localization of NS2 and NS3 proteins the Western blot analysis of nuclear and cytoplasmic extracts from BgDNV-infected BGE-2 cells was utilized. It was shown that NS2 protein was evenly distributed in cytoplasm and nucleus while NS3 was characterized by predominantly nuclear localization. It is worth mentioning that NS3 lacks any NLS sequences that could imply some other mechanisms are involved in rendering NS3 the ability to enter the nucleus.

Since BgDNV VP2 and VP3 proteins share a contiguous part of their aminoacid sequences with VP1 no approach using antibodies could be used to determine the individual localization of the corresponding proteins. To overcome this obstacle transient expression of each of BgDNV capsid proteins in COS-1 cells using GFP-fusion expression vector was used. It was demonstrated that VP1 and VP3 proteins were localized in nuclei while VP2 protein was detected both in the nuclei and the cytoplasm.

P20-125
Role of TRP domain in TRPV1 functionality

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TRPV1 (Transient receptor potential vanilloid 1) is a member of the TRP channel family activated by physical and chemical stimuli. TRPV1 functions as a non selective tetrameric cation channel with high permeability to calcium ions. Each subunit shows a topology of six a-helical transmembrane segments with a pore region between the fifth and sixth segment. The cytoplasmatic N- and C-termini contains several residues involved in the modulation of channel activity. Specifically, the C-terminal region contains the TRP domain (Glu684-Arg721), a highly conserved sequence in the TRP channels family. This region is a molecular determinant in the functional coupling of the channel. To further understand the role of this region in the protein functionality we performed a site directed mutagenesis strategy on a non functional TRPV1 chimera, TRPV1-AD2 that contains the TRP domain of TRPV2. We carried out the study of the mutated chimeras in transitory transfected HEK cells. First of all, we examined the expression level and the presence in plasmatic membrane for the mutated channels. Afterwards, the response of the mutated chimeras to capsaicin, depolarizing voltages and high temperature was also studied using the Patch Clamp technique. Taking together, our data suggest that the TRP domain region is critical for the functional coupling of the activating stimuli. Particularly, we found that alterations in the TRP domain affected the energetic of channel opening. These results demonstrate that the preservation of this region is essential for the correct functionality of TRPV1.

P20-126
Mature CpdB proteins from *Escherichia coli* and *Yersinia intermedia* with hydrolytic activity on the bacterial regulator cyclic 3′,5′-diadenylate or c-di-AMP

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CpdB is a 2′,3′-cyclic nucleotide 2′-phosphodiesterase synthesized as a precursor targeted to the periplasm of gram-negative (GN) bacteria. It contains metallophosphatase and 5′-nucleotidase, C-terminal superfamily domains, being about 25% identical to bacterial 5′-nucleotidase (UshA). During the cloning of a putative c-di-AMP hydrolase (c-di-AMPase) from a gram-positive (GP) organism, and upon its expression in GN *E. coli* BL21 cells, c-di-AMPase activity not attributable to the recombinant protein, but to the expression host, was detected. Since c-di-AMP is known only in GP bacteria, the identification of the *E. coli* c-di-AMPase was pursued. A ≍ 66-kDa band partly purified from BL21 cells was associated to this activity, and its peptide-mass fingerprint (PMF) pointed clearly to CpdB. Therefore, the coding sequence of its mature form (CpdB 20-647) was cloned from BL21 genomic DNA and over-expressed as a GST fusion that adsorbed to GSH-Sepharose, allowing the recovery of GST-free mature CpdB by in-column proteolysis. Recombinant CpdB hydrolyzed both 2′,3′-cAMP and c-di-AMP. The final product of c-di-AMP hydrolysis was 5′-AMP possibly via pApA. Bacteria from the GN *Yersinia* genus are known to contain also periplasmic CpdB. The periplasm of *Y. intermedia* cells was used as the starting point for the partial purification of hydrolytic activity on 2′,3′-cAMP, which yielded a ≍ 66-kDa protein band identified as CpdB by its PMF and associated also with c-di-AMPase activity. To our knowledge, this is the first report of c-di-AMP hydrolysis by GN species, and by bacterial proteins that do not contain GGDEF and DHH/DHHA1 or EAL domains, like the known hydrolases of c-di-AMP or c-di-GMP in GP or GN bacteria. (Junta de Extremadura, GR10133, cofinanced by FEDER).

P20-127
In vivo properties of mutant FUS variants

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Recently a concept of FUS or TDP-43 proteinopathies, i.e. disorders characterized by pathological alterations in the metabolism of these two DNA/RNA-binding proteins has emerged. Here belong a number of neurodegenerative conditions, first of all, ALS and FTDU. The proteins display striking structural similarities, where FUS is 'reverse' in its domain structure to TDP-43 protein. Both proteins were shown to have 'prion-like' domains, mapping to N-terminus of FUS and C-terminus of TDP-43. It is well established now that histopathological inclusions in the affected regions of patients with ALS-TDP are enriched with truncated ~25 kDa C-terminal fragments of TDP-43. In our *in vivo* study we aimed to assess the properties of N-terminal fragment of FUS, structurally equivalent to 25 kDa C-terminal TDP-43 fragment, compared to other FUS protein isoforms. Upon expression of GFP-tagged proteins in neuroblastoma cell line we found that C-terminal fragments of FUS form intracytoplasmic inclusions, principally different from those formed by full-length mutant protein variants. This protein form assembled in structures other than stress granules, and, using a number of markers, these inclusions were further characterized as possessing properties of aggresomes, i.e. protective cellular structures sequestering potentially deleterious misfolded proteins. Consistently with the presence of prion-like domain in truncated FUS, we also showed its ability to recruit other FUS protein isoforms into aggregates using the same model system. Thus, this model molecule might be useful for getting further insights into the role of different domains of DNA/RNA-binding proteins as well as for creation of *in vivo* models of FUS proteinopathy.

P20-128
Structural mechanism of cognate DNA recognition by the BfiI restriction enzyme

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Restriction endonuclease BfiI recognizes and cleaves the asymmetric DNA sequence 5′-ACTGGG-3′ downstream of the site independently of Mg-ions. BfiI is evolved as a fusion of two domains: the C-terminal DNA binding domain similar to DNA binding domains of EcoRII and B3 family of plant transcription factors, and the N-terminal catalytic domain, which is similar to the Nuc nuclease of *S. thyphimurium*. We have solved the crystal structure of the BfiI DNA binding domain in complex with the specific DNA to reveal the mechanism of the specific DNA recognition (manuscript in preparation). Superposition of the apo-

BfiI structure with the DNA bound C-domain suggests that BfiI must change its conformation to accommodate the scissile phosphate in the active site. To get a glimpse on the structural changes occurring upon BfiI binding to cognate DNA we have performed the X-ray small angle scattering (SAXS) measurements of apo and DNA bound BfiI. Ab initio shape determination as well as rigid body modeling using the crystallographic data suggest that apo BfiI retains the similar conformation in the solution as in a crystal, whereas DNA bound BfiI shows a conformational flexibility. The truncated heterodimer of BfiI which lacks one of the two DNA-binding domains was constructed in order to simplify the system for SAXS experiments.

P20r-129
Crystal structure of the Fra a 1E allergen, a major regulator of the flavonoid pathway in strawberry fruits

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START/Bet v 1 proteins are widespread in nature and participate in different processes such as lipid transport and steroid hormone synthesis. However, in most cases their mechanisms of action are still poorly understood. Recently, our lab has characterized the Fra a 1E allergen, a member of the START/Bet v 1 protein family in strawberry, as an essential component in flavonoid biosynthesis. To further characterize Fra a 1E biological roles, here we present its crystal structure to a 2.2 Å resolution. Fra a 1E structure consists of a seven- stranded antiparallel b-sheet and three a-helices that reveal a large hydrophobic cavity, which could be able to enclose different physiological ligands. The presence of a flexible b3-b4 loop indicates that ligand binding to the hydrophobic cavity could induce conformational changes in the protein that stimulate interaction with other proteins and the formation of stable complexes. The identification of these features in the crystal suggests that this protein is likely activated by binding of secondary metabolites to its central cavity. Thus, our results support the role of Fra a 1E in the development of color and flavor in strawberry fruits by regulating the flavonoid pathway, an important finding of biotechnological relevance.

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P20r-130
Using bacterial inclusion bodies to screen for amyloid aggregation inhibitors

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The amyloid-beta peptide (Abeta42) is the main component of the inter-neuronal amyloid plaques characteristic of Alzheimer's disease (AD). The mechanism by which Abeta42 and other amyloid peptides assemble into insoluble neurotoxic deposits is still not completely understood and multiple factors have been reported to trigger their formation. In particular, the presence of endogenous metal ions has been linked to the pathogenesis of AD and other neurodegenerative disorders.

Here we describe a rapid and high-throughput screening method to identify molecules able to modulate amyloid aggregation. The approach exploits the inclusion bodies (IBs) formed by Abeta42 when expressed in bacteria. We have shown previously that these aggregates retain amyloid structural and functional properties. In the present work we demonstrate that their *in vitro* refolding is selectively sensitive to the presence of aggregation-promoting metal ions, allowing the detection of inhibitors of metal-promoted amyloid aggregation with potential therapeutic interest.

Because IBs can be produced at high levels and easily purified, the method overcomes one of the main limitations in screens to detect amyloid modulators: the use of expensive and usually highly insoluble synthetic peptides.

P20-131
Use of principal component analysis and molecular docking to identify novel selective plasmepsin II inhibitors

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Plasmepsin II (PlmII) is an aspartic protease involved in the initial steps of the hemoglobin degradation pathway, a critical stage in the *Plasmodium falciparum* life cycle during human infection. However, most of the PlmII inhibitors obtained through structure-based ligand design have generally shown a low selectivity towards the human related protease Cathepsin D (hCatD), which is a notable drawback to their use as antimalarial drugs. Here, we presented a novel in silico approach based on the combined use of principal component analysis and molecular docking to identify selective inhibitors of PlmII. We searched unique conformational states of PlmII that can not be adopted by the human aspartic proteases: Cathepsin D, Renin and Pepsin by comparing the conformational subspaces sampled by these proteins along molecular dynamic simulations of 1.2 μs. Specific conformations along the flap opening-siding mode of PlmII that can not be sampled by the human counterparts were identified. The specific conformations were used to perform virtual screening experiments and proposed putative PlmII selective-inhibitors. The hCatD was also targeted to exclude non-selective compounds. The first five ranked inhibitors, with inhibition constants (Ki) values in the μM-nM range, target a cryptic flap interior pocket formed by the residues M75, V82, V105, T108, and Y115 which is only exposed in the PlmII specific conformations. The inhibition assays showed that the inhibitors bind better PlmII than hCatD in a range from 70 to 100-fold of their Ki values.

P20-132
Mapping the functional domains of plant HEN1 small RNA methyltransferase

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Non-coding small RNAs such as miRNAs, siRNAs and piRNAs are essential for post-transcriptional gene regulation in eukaryotic organisms including humans. Biogenesis of plant miRNAs and

siRNAs or animal piRNAs and Ago2-loaded siRNAs involves modification of the 2′-OH group at the 3′-terminal nucleotide. The best characterized member of small RNA 2′-*O*-methyltransferases family HEN1 from Arabidopsis thaliana catalyzes methyl group transfer to miRNA/miRNA* and siRNA/siRNA*. Structural analysis of this large protein revealed two putative double-stranded RNA-binding domains (dsRBD1 and dsRBD2) divided by a La motif-containing domain (LCD) in N-terminal part, the central peptidyl prolyl cis-trans isomerase-like domain (PLD) and C-terminal methyltransferase domain (MTD). Kinetic and functional study of series of the HEN1 deletion variants have established that MTD is involved in methyl group transfer, whereas dsRBD1 is crucial for HEN1 interaction with the double-stranded RNA substrates. The dsRBD2 and PLD may play appreciable role in forming contacts with other proteins involved in miRNA biogenesis, the experimental validation of this hypothesis is underway. The work was supported by grant from the Research Council of Lithuania MIP-028/2012.

P20-133
Structure of the plakin domain of plectin

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Plectin is a member of the plakin family of proteins that cross-links components of the cytoskeleton and link them to membrane-associated structures, such as hemidesmosomes.

Plectin has a multi-domain structure. The N-terminal region contains a conserved domain terme the plakin domain that consists of an array of nine Spectrin Repeats (SR1 to SR9) arranged in tandem and a Src-homology 3 (SH3) domain inserted in the central SR5 [1].

We have combined x-ray crystallography and SAXS to elucidate the structure of the plakin domain of plectin. Here, we present the crystal structure of several fragments of the central (SR3-SR6) and C-terminal region (SR7-SR9) of the plakin domain, that together cover the region SR3-SR9. Each SR consists on three helices (A, B y C) connected by short loops and packed in a helical bundle with a up-down-up topology. Adjacent SRs are linked by a continuos helix formed by the fusion of the helix-C of the N-terminal repeat and the helix-A of the C-terminal repeat. Yet there is no conservation in the relative orientation of adjacent SRs. The SH3 domain of plectin shows the canonical SH3 fold, but exhibits alterations in its putative Pro-rich binding-site suggesting that this domain does not bind to Pro-rich motifs [2].

Moreover, the SH3 binding-site is occluded by intramolecular contacts with the SR4. Residues that participate in the SR4-SH3 interaction are conserved in other members of the plakin family. The structure of the plakin domain of plectin serves as a structural model for other plakins.

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P20-134
Molecular-genetic analysis of the structural determinants and primary functions of eukaryotic ribosomal proteins L16 and L19 in *Saccharomyces cerevisiae*

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The way in which protein and RNA macromolecules perform their roles in the eukaryotic ribosomal complex can be investigated using the genetic amenable organims *Saccharomyces cerevisiae*. The eukaryotic ribosome is larger and more complex than its prokaryotic counterpart, containing additional expansion segments of rRNA (ES), novel r-proteins (Rp) and Rp-extensions that likely correlate with the higher complexity and regulation of eukaryotic translation. The recently resolved crystal structures of the eukaryotic ribosome predict new molecular interactions amongst Rp and ES, the functional significance of which is unknown [1,4].

Mutations altering the dosage, structural integrity or on-time assembly of Rps into pre-ribosomal particles impair specific steps of the pre-rRNA processing pathway, r-subunit biogenesis or 80S function, and are cause of ribosome heterogeneity, leading to changes in the patterns of protein synthesis, cell growth and differentiation [5,6]. Moreover, haploinsufficiency or Rp-mutation is associated with tumour predisposition in many organisms, and with human 'ribosomopathies' [2,7].

To unveil the primary roles in ribogenesis and translation of the essential L16 and L19 proteins of the 60S r-subunit, we generated random mutations in one of the two corresponding gene-paralogues in *S. cerevisiae* and selected partial loss-of-function and lethal mutants. L19 is predicted to map at the polypeptide exit tunnel of the 60S, with its α-helix at the C-terminal domain contacting the ES6 of 18S rRNA as part of the new eukaryotic intersubunit bridge eB12. L16 contacts rRNAs 25S and 5.8S and maps near to the ribosomal stalk, in a region interacting with translation initiation factors [1,4]. Molecular and cellular analysis of the L16 and L19 mutant phenotypes, along with bioinformatic predictions, allows correlation of defects *in vivo* with alterations of molecular interactions predicted from the yeast ribosome crystal structures. Data will be presented on the location of a set of L16 and L19 mutations, the pre-rRNA processing and translational defects of the mutants, and the impact of mutations on the GCN4 specific mechanism of translational regulation [3].

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P20-135
Initial biochemical and functional characterization of a recombinant 5′-nucleotidase from the plant pathogen *Xylella fastidiosa*

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Xylella fastidiosa is a Gram-negative bacterium that forms a biofilm inside the xylem vessels of susceptible plants and causes several economically relevant crop diseases. Here, we report the initial biochemical and functional characterization of the 5′-nucleotidase Xf5′-Nt from *X. fastidiosa*. The 5′-nucleotidases are a family of enzymes that catalyses either the hydrolysis or the transfer of esterified phosphate in the 5′ position of nucleoside monophosphates and regulate cellular nucleotide and nucleoside levels. The ORF *Xf*1808, which encodes the Xf5′-Nt protein, was cloned into the pET29a vector and overexpressed in *Escherichia coli*. The protein purification was achieved with a single affinity chromatographic step. The identity and the contents of secondary structures of the purified protein were confirmed by mass spectroscopy and circular dichroism, respectively. Initials kinetics assays using pNPP (*p*-nitrophenyl phosphate) as substrate confirmed the nucleotidase activity and revealed the requirement of divalent metals for full enzyme activity. In addition, we investigated the involvement of Xf5′-Nt in *X. fastidiosa* biofilm formation which is the structure that blocks the xylem and causes the onset of disease. Using polyclonal antibodies against Xf5′-Nt we demonstrate that Xf5′-Nt is differentially expressed during bacterial biofilm formation and planktonic growth. Our results show that the dephosphorylation network catalyzed by 5′-nucleotidases may play an important role for the bacterial biofilm formation and cell signaling adding new insights into bacterial nucleotides metabolism and pathogenicity.

P20-136
Identification of potential phosphorylation sites of actin-interacting protein 1

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Protein phosphorylation is the most common and important reversible posttranslational modification. Phosphorylation of any site on a given protein can modulate the function of that protein, therefore identification of possible phosphorylation sites play an important role in characterization of proteins. Actin-interacting protein 1 (Aip1), also called WD-repeat protein 1 (WDR1) was found phosphorylated in leukemia, lung and gastric cancer. It is also known that is involved in disassembly of actin filaments in conjunction with ADF/cofilin family proteins[1]. Actin cytoskeletal dynamics and reorganization play a crucial role in a variety of cellular processes, including cell migration, cytokinesis, endocytosis and morphological changes[2]. Here, we report the identified phosphorylation sites of Aip1 both *in vitro* and *in vivo*. Aip1 gene was successfully cloned both in a prokaryotic and eukaryotic expression vector. Our preliminary results indicate that Aip1 protein is phosphorylated by a protein tyrosine kinase (PTK) *in vitro*. To identify the potential tyrosine phosphorylation sites we used two phosphoprediction softwares available online. According with the results obtained we designed two single mutants and the double mutant, in which we mutated the corresponding tyrosine residues in phenylalanine residues. All three mutant proteins and the wild-type protein were subjected for phosphorylation by *PTK*, both *in vitro* and *in vivo*. Our results indicate that at least one of tyrosine residues studied represents the major phosphorylation site of Aip1 protein. Further studies will indicate if and how this posttranslational modification is affecting Aip1 function or localization. Acknowledgment This work was financially supported by POSDRU 89/1.5/S/60746.

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P20r-137
Natural genetic transformation in *H. pylori*: DprA interactome role

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Helicobacter pylori, the only bacterial pathogen classified as a human carcinogen by the WHO, displays an amazing genetic variability. The molecular mechanisms underlying its genomic plasticity have being partially assigned to error-prone DNA repair systems, to high levels of DNA replication errors and to efficient homologous or homeologous recombination. Intensive genetic exchanges between strains involved during natural transformation. This mechanism can also be in part responsible to the appearance and transmission of new antibiotic resistances, a phenomenon widely observed in clinical studies. Natural transformation has been particularly studied in the pathogen *S. pneumoniae*. Relatively little information is available for *H. pylori*. However, it points to important difference with respect to *S. pneumoniae*. For example, in *H. pylori* competence is constitutive unlike in *S. pneumoniae* where it is highly regulated. *H. pylori* has only one SSB without a specialised SSB for transformation. HpRecA is highly and constitutively expressed. Transformation is enhanced in AddAB-deficient strains, and DprA inactivation completely abolishes transformation. In *S. pneumoniae* these two key proteins, DprA and RecA, are induced during transformation and are required through a subtle network of interactions for handling the incoming DNA once within the cell. Little is known at the molecular level about the choreography and timing of the interactions. We start to address their roles in *H. pylori*, by the combination of biochemical, biophysical and structural approaches *in vitro* and in silico, completed with phenotypic analyses *in vivo*. We have solved the crystal structure of the central domain of H.pDprA that we can now compare to the two other known ones: *S. pneumonia* and *R. palustris*. We seek to analyse the interaction surfaces of DprA with its different partners and with DNA to propose mutants for which the phenotypic impact on transformation will be studied determined *in vivo*. We will sec-

ondly focus on the structure-function analysis of the C-terminal extra-domain of H.pDprA.

P20m-138
In silico analyses of PII – disintegrins interaction with αIIbβ3, αvβ3 and α5β1 integrin family

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PII-disintegrins, cysteine-rich polypeptides broadly distributed in the venoms of geographically diverse species of snakes that modulated the adhesive functions of αIIbβ3, αvβ3 and α5β1. The objective of this study is analyze the selectivity of interaction between the disintegrins and integrins receptors. For that, we performed an *in silico* analyses using disintegrins, and the structures from those integrins. Initially, we constructed the databank with all disintegrins deposited in NCBI and SWISS-prot databank to construct the alignment and a phylogeny of them to observe the pattern of the structures. According with the alignment and phylogeny results, it possible to observe four distinct groups that posses the high similarity among them. From those groups, we select two disintegrins of these four groups: group 1 (trimestatin and flavoridin), group 2 (jarastatin and salmosin) group 3 (kistrin and jararacin), group 4 (albolatin and mojastin) and whose were used to construct the complexes with integrins αIIbβ3, αvβ3 and α5β1. The 24 complexes constructed were minimized with the GROMACS program. With these complexes, we analyze the interaction with the webservers PISA, protein interaction calculator (PIC) and use the GROMACS program to analyze the short range of Lennard-Jones and Coulombic energy that represent the interaction energy of the system. Analysis of the complexes with α5β1, the group 1 and group 2 and kistrin present lowest values of interaction energy and the number of iterations is greater than others. And the others two groups present a low interaction with α5β1. αIIbβ3 complexes show the jararacin, but not kistrin, group 1 and group 4 present lowest values of interaction energy and the number of iterations is greater than the group 2 and kistrin. And the αvβ3 complexes show group 2 with the high interactions values than others followed by group 1 and group 4. These results should be useful for designing and developing more effective drugs for controlling pathologies that involves these receptors and understanding the interaction mechanisms of integrins functions.

P20-139
Identification of estrogen responsive element in the human immunodeficiency virus (type 1) long terminal repeat

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Previous studies proposed a role of estrogen for HIV-1 replication through estrogen receptor alpha (ERα) by enhancing Sp1 DNA-binding and transcriptional activity. In view of the fact that estrogen can modulate expression through ER in both estrogen responsive element (ERE)-dependent and ERE-independent modes, we searched the nucleotide sequence of the HIV-1 long terminal repeat (LTR) to identify putative ERE. The analysis was performed by the informational spectrum method (ISM), a virtual spectroscopy method for structure/function analysis of nucleotide and protein sequences. Potential ERE was identified at position -92 through -63, showing sequence and structural similarity to the ERE consensus sequence. Possible consequences of existence of ERE-like sequence in HIV-l LTR on viral latency and short-term use of steroids in HIV-related diseases are discussed.

P20-140
The 14-3-3 protein binding affects the conformation of both the N- and C- terminal domains of phosducin

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Phosducin (Pdc), a regulatory and highly conserved 30 kDa acidic phosphoprotein, is present in a variety of cell types, with especially high expression in retinal photoreceptors and pinealocytes. Pdc regulates the G-protein signaling by competing with Gα for binding to Gβγ subunits. The ability of Pdc to bind to Gβγ depends on its phosphorylation state as only unphosphorylated Pdc binds Gβγ tightly. Phosphorylation of Pdc within the N-terminal part inhibits its binding to Gβγ through unclear mechanism involving the interaction between Pdc and the regulatory 14-3-3 protein. In this work, we studied interactions between phosphorylated Pdc and 14-3-3 using analytical ultracentrifugation and time-resolved fluorescence spectroscopy. Our results show that Pdc and 14-3-3 form a stable complex with 1:2 molar stoichiometry and that the 14-3-3 protein binding significantly affects the structure of both the N- and C-terminal domains of Pdc. This work was funded by the grant P305/11/0708 of the Grant Agency of the Czech Republic.

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P20-141
Novel calcium regulation of actin-binding activity of smooth muscle myosin light-chain kinase

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Smooth muscle myosin light chain kinase (SmMLCK) exhibits not only kinase activity, for example, phosphorylation of the

myosin regulatory light chain, but also non-kinase activity, for example, actin-binding. We have previously reported that calcium-calmodulin (Ca-CaM) regulate the actin-binding activity of SmMLCK. In this study, we investigated the effects of two different CaM-binding sites on actin-binding and bundling properties. We generated a construct of kinase-dead SmMLCK that had lost the CaM-binding site (M13: 1003R-1021S) for kinase activation using by Takara's pCold expression vectors that cold shock expression system for high purity, high yield protein production. It seemed that the mutant lost the regulation by Ca-CaM, but the regulation still remained even though it had lost the M13 site. Therefore, we reanalyzed another CaM binding site on SmMLCK using by the CaM Target Database. The second CaM-binding site (X site: 749K-754K) was identified at the upstream of the N-terminus of the M13 site. We examined the CaM-binding properties of the M13 and X peptides using by the CaM magnetic beads assay and Biacore's SPR technology experiment. The result showed that the X site bund CaM. To confirm the effect of the X site on actin-binding activity of SmMLCK, we generated a double mutant with a mutation that abolished CaM-binding at the X and M13 sites. The mutant of SmMLCK could not bind CaM and had therefore completely lost its ability to regulate actin-binding activity through Ca-CaM. This second CaM binding site also affected the actin-bundling activity. Thus, our results indicate that the second CaM-binding X-site was involved in the regulation of non-kinase activity by calcium-CaM through actin. The Ca-CaM regulation of the non-kinase activity of SmMLCK may be required for dynamics of the acto-myosin system important for muscle contraction and migration.

P20-142
DNA recognition by the methyl-specific endonuclease McrBC

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DNA methylation is a covalent DNA modification that is abundant in most forms of life. Modified cytosine variants 5-methylcytosine (5mC) and the recently discovered 5-hydroxymethylcytosine (5hmC) are involved in the epigenetic regulation of gene expression in eukaryotes. The discovered role of aberrant methylation in such processes as cell differentiation and carcinogenesis has stimulated development of new methods for DNA methylation profiling. Methyl-directed restriction endonucleases (enzymes that recognize and cleave DNA target sequences with 5mC and/or 5hmC modifications) discovered in various bacterial strains are promising molecular tools for the studies of 5mC and 5hmC distribution in eukaryotic genomes.

We have recently solved the crystal structure of the N-terminal DNA-binding domain (McrB-N) of the methyl-specific endonuclease McrBC from *E. coli* [Sukackaite, R. et al. (2012) The recognition domain of the methyl-specific endonuclease McrBC flips out 5-methylcytosine. *Nucleic Acids Res.* doi:10.1093/nar/gks332]. In the crystal structure McrB-N flips out the 5mC base from the DNA duplex and positions it within a binding pocket. The pocket walls are made by the side chains of the conserved residues W49, L68, Y117, Y64, S120 and A59. The pocket size is sufficient to accommodate both 5mC and 5hmC bases, therefore the McrB-N domain has no selectivity for DNA sequences with either 5mC or 5hmC modifications.We report here mutational analysis of McrB-N aimed at increasing its selectivity for either 5mC or 5hmC bases.

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P20-143
Protein–protein interactions in phage T4 primosome

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DNA replication is a process essential in all known life forms. Unfaithful copying of the DNA template results in mutations that change genetic blueprint of the cell and lead to genetic disorders or cancer development. Primosome is a module of the replication complex, required for the initiation of the discontinuous synthesis of the lagging DNA strand. Phage T4 primosome is composed of primase gp61, helicase gp41, single stranded DNA binding protein gp32 and helicase loading protein gp59. Single stranded DNA binding protein gp32 seems to play an essential role in the coordination of the phage T4 lagging stand synthesis. It is thought that gp32 interacts with several replisome proteins; however structural details of the interactions are limited. In this study we aimed to identify the gp32 interaction partners through the pull down assay and map gp32-replisome protein interactions by cross-linking. We predicted the gp32 region presumably involved in the protein-protein interactions and analysed the importance of this region for gp32 interactions with replisome proteins by mutational analysis.

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P20-144
Tuned magnetic nanoparticles for studying surface-exposed proteins in bacterial cells

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The bacterial cell envelope is a highly structured multi-layer meant to assure cell integrity and protection from environmental adversities while supporting in-out trafficking of nutrients and wastes. Surface proteins localized in the outer membrane participate in interactions with the environment, such as sensing the chemical and physical conditions of surroundings and transmitting appropriate signals to cytoplasm. These functions include adhesion to and, when possible, invasion of physical and biological supports (e.g. host cells for pathogens); as well as transport of nutrient molecules. Given these essential roles in bacterial life and in pathogenicity, identification and characterization of envelope proteins may lead to better explain the relation of bacteria with the surrounding environment. We here describe a new and specific technique for magneto-capturing of surface-exposed proteins in intact bacterial cells. This method is based on the use of carboxymethyl-dextran coated magnetic nanoparticles activated so to be able to establish covalent bonds with exposed lysine groups in proteins. Given their chemical composition, size (average diameter = 80–90 nm), and negative charge, these NPs were expected to be atoxic for bacterial cells, as they are for eukaryotic cells. This approach greatly improves sensitivity and specificity of previous methods such as surface shaving with proteases. This techniques of magneto-separation of cell envelope fragments from the soluble cytoplasmic fraction also allows the identification of the captured proteins, and that of neighboring ones. The magneto-capture procedure is simple, safe, and rapid, and appears well-suited for envelope studies in highly pathogenic bacteria.

P20-145
FHIT and HINT1 proteins – catalytically promiscuous enzymes

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The system of regulation by oxidation-reduction of disulfide groups composed by thioredoxins (Trx) and thioredoxin-reductases (TR), is a key point of control in many cellular processes. Some TRs use NADPH as source of reducing power (NTR). Canonical NTRs contain a flavin (FAD) and a disulfide group as redox cofactors, operating in an intramolecular electron transfer chain from NADPH to the disulfide at the active site. The catalytic mechanism of NTRs involves a large interdomain rotation movement between two configurations. This conformational change is probably the rate-limiting step in catalysis.

Plants possess a particular type of chloroplast NTR (NTRC), resulting from the fusion of a NTR module with a Trx module, and thus containing an additional disulfide group. Among other functions, NTRC is involved in protection against oxidative stress, using as substrate 2-Cys peroxiredoxins (2-Cys Prx), a family of antioxidant proteins with disulfide groups involved in peroxide detoxification.

A relevant question is whether the conformational change mechanism of canonical NTRs also occurs in bimodular NTRC. A kinetic study carried out comparatively with NTRC, the truncated NTR module of the enzyme and one archetypical plant NTR (NTRB), has shown that NTRC maintains the conformational change associated with catalysis, and also shows additional dynamic behaviors associated with the electron transfer to the Trx module. Moreover, these additional dynamic features are altered in the presence of 2-Cys Prx. NTRC shows structural constraints that fix the Trx module in positions with different efficiency for electron transfer, and the presence of 2-Cys Prx shifts the conformational equilibrium of the Trx domain to a specific fixed position, which is not the most efficient.

P20r-146
Electron transfer reactions and dynamics of plant chloroplastic NADPH-dependent thioredoxin-reductase (NTRC)

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P20r-147
Fungal extracellular ribotoxins as natural insecticidal agents

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Fungal ribotoxins were discovered almost 50 years ago as extracellular ribonucleases (RNases) with antitumoral properties. It was quite obvious from the very beginning, however, that fungi were not secreting these lethal toxins with the purpose of combating mammalian cancer. The real biological function of these toxic proteins has therefore remained elusive. The discovery of the ribotoxin HtA, produced by the invertebrates pathogen *H. thompsonii*, revived the old proposal that being insecticidal would be their long searched natural function. Unfortunately, HtA is rather unique among all ribotoxins known in terms of sequence and structure similarities. Thus, it was intriguing to answer to the question of whether HtA is just an exception or, on the contrary, the paradigmatic example of the natural function of ribotoxins. The work presented uses HtA and α-sarcin, the most representative member of the ribotoxins' family, to show the strong insecticidal action of ribotoxins against insect larvae and cells.

P20-148
Unravelling the cellular function of the endogenous carboxypeptidase inhibitor, latexin

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Metallocarboxypeptidases (MCPs) are unique zinc-dependent enzymes that catalyze the breakdown of the amide bond at the C-terminus of peptide and protein substrates, they have jumped from being mere contaminants in animal pancreas powders to be key players in cellular and molecular processes. A variety of MCPs have been linked to diseases: acute pancreatitis, type 2 diabetes, Alzheimer's Disease, various types of cancer, fibrinolysis and inflammation. To date, the only specific endogenous inhibitor present in mammals is latexin, a 25 kDa protein, that shares no structural similarity with any other known carboxypeptidase inhibitor. Latexin is widely expressed in humans, expression is high in heart, prostate, ovary, kidney, pancreas, colon and moderate in brain. It has been found to inhibit mouse stem cell populations and lymphoma cell proliferation, demonstrating its potential role as a tumor suppressor. Latexin has also been shown to play a role in inflammation, aging and longevity. Despite the biochemical function of latexin as carboxypeptidase inhibitor is well demonstrated, its biological function remains unclear. Therefore, the identification of proteins and factors that interact with latexin in mammal cells is of great interest. For

characterizing the latexin interactome, strep-tag purification combined with proteomic quantitative analyses is used in different cancer lines expressing latexin such as epithelial carcinoma, pancreatic carcinoma or breast cancer. In addition, knockout of latexin in these lines would help to understand its function in the cell. Biomedical and functional analyses could constitute a key point to elucidate latexin function at the intersection of stem cells, aging, and cancer in ways that come full circle with events in embryogenesis and tissue regeneration.

P20-149
How important is the arrangement of conserved domains in histidine kinase?

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The histidine kinase DosS and DosT are essential for survival of *Mycobacterium tuberculosis* under low oxygen condition in a dormant state and responsible for recognition of hypoxia by the two-component regulatory systems. Both DosS and DosT consist of N-terminal sensing core composed of two GAF domains and C-terminal KC with a HisKA domain and ABD domain without any transmembrane or extracellular motifs. The first GAF domain has a heme for detecting oxygen tension while the function of the second GAF domain is not known yet. Here, we report the ABD crystal structures of the DosS and DosT. Their fold is highly similar to other ABD structures, such as PhoQ and CheA, but the ATP-lid loop of DosS is much shorter than others. The ABD structures show two-layered *a*/*b* sandwich fold, that composed of five-stranded *b*-sheet and three *a*-helices. The ABDs are relatively compact and does not have ATP lid motif. Crystal structures of the ABDs revealed that a short loop overlay putative ATP binding site and those were not desirable for ATP binding alone. KC dimerization is thought to be enough to induce ABD conformational change to be an active form in the presence of HisKA domain. However, KC does not exist as one stable active dimer form. Arrangement of additional second GAF domain in the N-terminus enforces the proper dimerization of KC. The second GAF domain not only enhances its kinase activity but also protract the phosphatase activity for phosphorylated response regulator, DosR. Hence, the second GAF domain plays roles for both enzymatic activities of KC and regulating function of the first GAF domain.

P20-150
Hint1 and Fhit enzymes form complexes with nucleoside 5′-O-phosphorothioates *in vitro*

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Hint-1 protein (histidine triad nucleotide binding protein) and Fhit protein (fragile histidine triad protein) belong to the histidine triad superfamily (HIT) of proteins, the enzymes which are known to be nucleotidylyl transferases and hydrolases. *In vitro* Hint1 and Fhit have shown substrate specificity towards many compounds containing phosphate linkages of different type as phosphoramidates (P-N bond), phosphates (P-O bond), phosphorothioates (P-S bond) and phosphorofluoridates (P-F bond). We have found that Hint1 can hydrolyze P-S bond in adenosine 5′-*O*-monophosphorothioate (AMPS) at the rate 0.2 nmol/min/μg, what is about 40-times faster than the Fhit enzyme does that. On the other hand, some phosphorothioate analogs of dinucleoside polyphosphates have been reported as inhibitors of enzymatic activity of the Fhit protein. Recently, the phosphorothioate modification of DNA has been identified in bacteria, therefore, the research of metabolism of phosphorothioate nucleotides has gained additional importance. We synthesized non-hydrolysable phosphorothioate dinucleoside derivative Br^5dU-3′-$_{PS}CH_2CH_2CH_{2PS}$5′-A, which contains 5-bromo-2′-deoxyuridine (Br^5dU), the residue allowing to make a covalent bond in nucleic acids-proteins complexes by means of photocrosslinking (at 300 nm). The compound consists of four diastereoisomers because of chirality at two phosphorus atoms, and only Sp-Sp isomer is fully non-hydrolysable. We have found that this isomer can form complexes with recombinant Hint1 and/or Fhit proteins. Moreover, similar complexes are formed with proteins present in cellular extracts obtained from A549 cells ($Hint1^+$, $Fhit^-$) or HEK293T cells ($Fhit^+$) instead of pure Hint1 and Fhit proteins. The formation of specific complexes with one of components of the proteins mixture indicate that direct interactions between the studied enzymes and phosphorothioate nucleosides are possible, also inside the cells. Moreover, because the Hint1 protein homologs are present in all forms of life, this enzyme seems to be a good candidate for carrying out the desulfuration of nucleoside 5′-*O*-phosphorothioates *in vivo*.

P20-151
Structure and assembly of an outer membrane channel for type IV pili in *Neisseria meningitidis*

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PilQ is a member of the secretin family, and forms a channel in the bacterial outer membrane for the passage of type IV pili. Here we present the structure of the PilQ dodecamer from *Neisseria*, in complex with the PilP lipoprotein, which is anchored in the inner membrane. Using NMR, we determined the structures of the periplasmic domains from PilQ: the N-terminus is shown to consist of two beta-type domains, which are unique to the type IV pilus-dependent secretins and have not been structurally characterised before. The central part of PilQ consists of two alpha/beta fold domains: we determined the structure of the first of these, and showed that it adopts a fold similar to that of related secretins. We also determined the structure of the entire PilQ dodecamer by cryoelectron microscopy, showing that it forms a large cage-like structure, sealed at both ends. Using NMR measurements, homology modelling and computationally-driven docking of domains into the electron microscopy density map, we were able to generate a model for most of the PilQ dodecamer. We also show that PilP binds to the first alpha/beta domain in PilQ and map the binding site for both proteins through measurements of NMR chemical shift changes. These results enable us to generate a structure for the complex of PilP bound to the alpha/beta domain, and hence reconstruct the PilQ:PilP dodecameric assembly. The results show that the PilQ multimer needs to disassemble substantially to accommodate the pilus fibre, and that the beta domains must play a role in gating access to the secretin chamber. We propose that PilP functions to stabilise the PilQ assembly during the transit of pili across the OM, and to form a link between the inner and outer membrane components of the type IV pilus biogenesis system.

P20-152
Pre-steady state kinetics of interaction of wild-type and multiple drug-resistant HIV protease with 1st and 2nd generation inhibitory drugs

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More than 2 million people die annually due to AIDS. Despite years of intense research, no ultimate cure to AIDS has been developed. Introduction of HAART dramatically increased life expectancy for HIV-infected patients. HIV protease (PR) is one of the key targets of HAART drugs. However, major problem in the HIV therapy is continuous emergence of drug-resistant viral species. These HIV variants escape drug pressure thus propagating infection and eventual development of AIDS. Basing on the analysis of crystal structures of escape mutants, 2nd-generation HIV PR inhibitors were developed. These, however, could not provide genetic barrier sufficiently high to stop the spread of HIV escape mutants, although slowed it considerably. Further studies are needed to design HIV PR inhibitors with higher genetic barrier to block development of drug resistance. It is unlikely, that this can be done using purely structural approach, and study of dynamics of interactions between mutant HIV PR and inhibitors can provide new information required for the higher-barrier inhibitor design. In our study, pre-steady state kinetic measurements of interactions between mutant and wild-type HIV PR and indinavir and darunavir as 1st and 2nd generation inhibitors revealed important differences in their mode of interaction with HIV PR. The data collected can be used for development of new PR inhibitors and for evaluation of potential drug candidates. A multifaceted comparative analysis between properties wild-type and mutant HIV proteases represent promising approach to the ultimate anti-HIV therapeutics.

P20-153
Spider toxin OtTx from *Oxyopes takobius* venom is the first member of a novel class of modular toxins

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Spider venom is a rich source of molecules of interest to both fundamental and applied science. From the venom of *Oxyopes takobius* (Oxyopidae), we isolated a novel antimicrobial and insecticidal polypeptide OtTx (108 amino acid residues). It can be referred to a new class of spider toxins since its primary structure can be split into two modules, the N-terminal linear and C-terminal cysteine-rich. This polypeptide combining neurotoxic and cytolytic properties may exhibit an interesting mechanism of action. We further obtained recombinant toxin OtTx and two its derivatives to perform structure-functional studies. Ot-amp (41 residues) is the linear N-terminal module of OtTx, which was synthesized chemically. In membrane-mimicking environment it was found to assume an amphipathic α-helix typical of cytolytic peptides. Ot-ick (59 residues; 5 S-S bonds) is the C-terminal module of OtTx that probably forms the inhibitor cystine knot (ICK) fold characteristic of spider neurotoxins. Full-length OtTx and Ot-ick were obtained by genetic engineering methods. Synthetic genes coding for the target polypeptides were produced, cloned into plasmid vectors and expressed in *Escherichia coli*. Ot-ick did not fold properly, a refolding procedure was therefore used to drive the polypeptide assume its native 3D structure. We tested the antimicrobial activity of OtTx and its derivatives on Gram-negative and Gram-positive bacteria. The insecticidal activity of the polypeptides was tested on flesh fly larvae. Comparison of OtTx with its derivatives sheds light on the novel modular toxin mode of action.

P20-154
'Amyloid-like' fibril formation driven by anionic lipid membranes: multiparametric fluorescence detection of lysozyme oligomeric intermediates

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Negatively-charged phospholipids have been suggested to trigger 'amyloid-like' fibril formation by several non-amyloidogenic proteins, e.g. lysozyme [1]. To elucidate the factors that govern the formation of these 'amyloid-like' fibrils, lysozyme was labeled with Alexa 488 (A488-Lz) and its interaction with POPC LUVs containing 20 and 30 mol% of POPS was studied using both steady-state and time-resolved fluorescence techniques. The variation of the mean fluorescence lifetime of A488-Lz as a function of the surface coverage of the liposomes was quantitatively described by a cooperative partition model. Briefly, it was assumed that monomeric lysozyme molecules partition into the bilayer surface and reversibly assemble into oligomers with N subunits ($N \geq 6$). The lifetime data was globally analyzed using the partition coefficients previously determined for A488-Lz by fluorescence correlation spectroscopy (FCS) [2] and by taking into account the electrostatic effects by means of the Gouy-Chapman theory. The oligomerization state of lysozyme was further assessed by evaluating the extent of energy migration (homoFRET) between membrane-bound A488-Lz. The variation of A488-Lz steady-state fluorescence anisotropy with its surface concentration in the membrane was adequately described only for $N = 6 \pm 1$ when the binomial distribution of fluorescently-labeled monomers among the oligomers was considered. Finally, the lipid-protein supramolecular complexes formed at a low L/P molar ratio were characterized by fluorescence lifetime imaging microscopy (FLIM). The average fluorescence lifetime of A488-Lz had a uniform spatial distribution on these structures, and its short value confirmed the aggregated state of lysozyme.

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P20-155
Thermodynamic analysis of the molecular interaction between the actin-binding domain of plectin and the β4 integrin subunit

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Integrins are a family of heterodimeric adhesion and signalling receptors made up of α and β subunits. The integrin α6β4 is a

receptor for laminins with preference for laminin-332.[1] In epithelia α6β4 is located at the basal layer in the hemidesmosomes that are junctional adhesion complexes that anchor epithelial cells to the basement membrane. At the hemidesmosomes α6β4 is connected to the cytokeratin network by two proteins of the plakin family: plectin and BPAG1e. The interaction between the integrin α6β4 and plectin is essential for the assembly and stability of hemidesmosomes. Disruption of the β4 integrin-plectin-binding interface is directly linked to the development of *epidermolysis bullosa* (EB). EB is an inherited disease characterized by fragility and blistering of the skin, associated with characteristic extracutaneous manifestations.[2] The crystal structure of the primary α6β4-plectin complex, formed by the first pair of fibronectin type III domains of β4 and the actin-binding domain of plectin was described by our group.[3] In order to better characterize this interaction, here we present a thermodynamic analysis of the molecular interaction between the actin-binding domain of plectin and the β4 integrin subunit. The site-specific β4-plectin interaction investigated exhibited non-linear van't Hoff behaviour. The entropic and enthalpic contributions as well as the standard heat capacity change were determined.

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P20-156
Structure characterization of Rana ridibunda liver arginase

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One of an important objects of modern biology is the clarification of the metabolism regulation mechanisms, and isoenzymes have a significant role in this issue. Nowadays, finally confirmed the existence of arginase 2 isoenzymes (ureotelic and non-ureotelic), however the fully characterization of their protein structure is not yet performed, especially features of the subunits bind. This study investigated the subunit composition in Rana ridibunda liver arginase, during the reversible inactivation by EDTA (ethylenediaminetetraacetic acid). It has been shown, that Rana ridibunda liver arginase equally inactivated 80% within pH 7.4–9.5 by EDTA during the hour. The inactivation process expressed pronounced temperature dependency also, the enzyme inactivation prohibited by 90% in 0°C. The purity of the studied enzyme does not affect the inactivation process by EDTA. Previously made by our investigation, it has been found by EPR (electron paramagnetic resonance) Spectroscopy, that the enzyme proincubation with Mn^{2+} are prohibited the inactivation by 55%, which can be explained by the existence of two types of bivalent Mn ions in Rana ridibunda liver arginase, which are differ in their surrounding protein composition. Bivalent Mn ions considered the real cofactor for studied enzyme, in addition to activation these ions stabilize the conformation of enzyme, regulate the PH optimum, impact on termostability, limited proteolysis by trypsin, also on other properties. The extract of Rana ridibunda liver arginase (centrifugation at 25 000 g) and partially purified arginase (25 000 g and Sephadex G-200), which previously inactivated for an hour by EDTA, are restoration their activity as the absence of the Mn^{2+} (37–40%), respectively, both in the presence of Mn ions (60–72%).

P20-157
The pH sensor of the plant K+ uptake channel KAT1 is built from a sensory cloud rather than from single key amino acids

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The uptake of potassium ions (K+) accompanied by an acidification of the apoplasm is a prerequisite for stomatal opening. The acidification (approximately 2–2.5 pH units) is perceived by voltage-gated inward potassium channels (Kin) that then can open their pores with lower energy cost. The sensory units for extracellular pH in stomatal Kin channels are proposed to be histidines exposed to the apoplasm. However, in the *Arabidopsis thaliana* stomatal Kin channel KAT1, mutations in the unique histidine exposed to the solvent (His267) do not affect the pH dependency. We demonstrate in the present study that His267 of the KAT1 channel cannot sense pH changes since the neighbouring residue Phe266 shifts its pKa to undetectable values through a cation–π interaction. Instead, we show that Glu240 placed in the extracellular loop between transmembrane segments S5 and S6 is involved in the extracellular acid activation mechanism. Based on structural models we propose that this region may serve as a molecular link between the pH- and the voltage-sensor. Like Glu240, several other titratable residues could contribute to the pH-sensor of KAT1, interact with each other and even connect such residues far away from the voltage-sensor with the gating machinery of the channel.

P20-158
Inhibiting RAS interactions using peptides; computational design and experimental validation

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Protein-protein interactions are essential in the shaping of normal and pathological behaviours in cells and are ubiquitous in Biology. Thus, modulating protein-protein interactions by means of small (chemical) agents holds then the key for future and novel therapeutic interventions. Although a major challenge, recent successes reported in the literature get us closer to this dream. Peptide-mediated interactions accounts for a large fraction of the interaction that occur inside the cell and thus peptides are gaining momentum as emerging agents for the modulation of protein-protein interactions. In this work, I will be presenting a novel, knowledge-based, computational, approach to design orthosteric peptides to inhibit protein-protein interactions. This particular methodology has been applied to a highly relevant target, RAS family, and the experimental results show that this method is able to derive peptides that target specifically the active form of RAS and thus prevent the interaction with cognate partners and a highly specific antibody. These results open a new avenue on peptide design and provide the basis for large-scale mining of current (structural) interactome.

P20-159
Novel insights into the function of enigmatic prokaryotic proteases TldD & TldE

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Escherichia coli TldD and *TldE* genes were at first isolated as implicated in CcdA antitoxin degradation and in the maturation of microcin B17. More recently other lines of evidence linked Tlds with the stability of carbon storage regulator CsrA and with the production of pyrroloquinoline quinone (PPQ) in several bacterial species. However, solved X-ray structure of *Thermotoga maritima* TldE does not include any possible protease site. Here we show that TldD and TldE proteins form a heterodimeric complex capable of cleaving modified microcin B precursors as well as other thiazole and oxazole heterocycle containing peptides. Efficiency of cleavage is proportional to the amount of modification. Thus, unmodified microcin B precursor peptides are not cleaved at all. TldD is proteolytically active subunit posessing known zincin protease active site motif (HEXXEX). Alanine substitutions of the conserved residues presumably involved in the Zn^{2+} ion coordination and catalysis resulted in the complex lacking specific activity. CsrA and CcdA peptides are not Tld substrates *in vitro* suggesting more complicated involvement of Tlds into the regulation of these systems. Bioinformatic search shows that Tld proteases are almost universally distributed in Eubacteria and Archea classes; the question remains about primary function of these enigmatic proteins.

P20-160
The crystal structure of lipase II, *Arabidopsis thaliana* DAD1-like seedling establishment-related lipase (AtDSEL) at 2.0 Å resolution

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Defective in another dehiscence 1 (DAD1) has been identified as a *sn*-1 specific lipase and several lines of evidences have suggested that the homologs of DAD1 play important roles in the regulation of various cellular processes, such as tissue growth, seed germination, stabilization of the thylakoid membrane structure, defense signaling, and seedling establishment. Recently, the cellular function of another class II protein, *Arabidopsis thaliana* DAD1-like Seedling Establishment-related Lipase (AtDSEL), has been proposed. *AtDSEL*-overexpressors showed the impaired seedling establishment under the condition without exogenous carbon source and was insensitive to 2,4-dichlrophenoxybutyric acid. These results indicate that the mobilization of storage oil for seedling establishment was blocked in the step of the β-oxidation in *AtDSEL*-overexpressors. We have determined the crystal structure of AtDSEL at 2.0 Å resolution. AtDSEL is an α/β hydrolase fold with a six-stranded parallel β-sheet flanked by five α-helices. The catalytic residues, Ser236, Asp302 and His339, and the residues forming the oxyanion hole are in positions very similar to those of other lipases. Despite structural similarity to other lipases, the AtDSEL adopts a unique dimer formation by an intermolecular β sheet. Functional studies demonstrate that the novel dimer interface is important for both activation and stability. Our structure will provide key information to understand about distinct biochemical activity and molecular function of these family proteins.

P20-161
Transport of the botulinum neurotoxin-associating protein, nontoxic nonhemagglutinin, across the intestinal epithelial cell layer

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Botulinum neurotoxin (BoNT) produced by *Clostridium botulinum* is a causative agent of food-borne botulism. In nature, the BoNT exists as a part of toxin complex by association with nontoxic proteins, nontoxic nonhemagglutinin (NTNHA) and hemagglutinins. Recently we demonstrated a possibility showing that the BoNT and NTNHA have evolved from common ancestral protein, although these proteins display very distinct functions; specifically, BoNT is toxic, whereas NTNHA is nontoxic. Additionally the crystal structure of the NTNHA published recently indicated that the BoNT and NTNHA shares quite similar structure consists of three domains. These findings imply that the BoNT and NTNHA share functional similarity. The BoNT can bind to intestinal epithelial cell and subsequently transport across the cell layer. In this study, we investigated whether the NTNHA molecule binds and permeates to the cell layer of the rat small intestine epithelial cell line IEC-6. NTNHA of serotype D strain 4947 (D-4947) was synthesized as recombinant protein in the *E. coli* system. The recombinant NTNHA (rNTNHA), as well as D-4947 BoNT, bound to the IEC-6 cell. Transport of the rNTNHA and BoNT was examined by using Transwell chamber system consists of two compartments separated by polycarbonate membrane on which IEC-6 cells were seeded. The rNTNHA and BoNT were poured to apical side of the cell layer, and then transported proteins were collected from basal side. As a result, the both were transported across the cell layer. This is the first finding that the NTNHA alone transports across the intestinal epithelial cell layer as far as we know. Currently, we attempt to clarify the responsible domain for the NTNHA transport across the cell layer using partial recombinant proteins of NTNHA.

P20-162
Structure of a novel M32 peptidase from disease-causing *Trypanosoma cruzi*

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The ability to avoid blue light radiation is very crucial for life sustainability of bacteria. Photoactive yellow protein (PYP) shows a ultra violet (UV) absorption spectrum, which matches with the wavelength dependence of the escape of the bacterium from potentially harmful blue-light radiation. Its response to the blue light is associated to the changes of the optical property of chromophore *para*-Coumaric acid (*p*CA) in the active site of PYP and display strong pH dependence in photocycle kinetic (1). Due to this observation, PYP has become an important example of rich model system for the study of Per-arnt-sim (PAS) domain

signaling and a soluble bacterial light sensor. However, the mechanism of pH dependence in the photocycle kinetics of PYP is still in dispute, in which some experimental and theoretical studies are contradictive. Therefore, a comprehensive *p*Ka determination is crucial in order to clarify the mechanism. In this study, we successfully determine all individual protonation states of titrable groups in the active site of PYP based on heteronuclear NMR chemical shift. To achieve that goal, we utilize a novel NMR approach to sequence-specifically follow all tyrosine side chain protonation states (2). At the same time, we also develop a simple and cheap labeling strategy to determine the pKa of arginine in PYP. These findings provide the insight into the electrostatic interaction in the active site of PYP and resolve the ambiguity of the pH-dependence in the photocycle kinetic

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P20-163
Structural systems biology: combining structures and interactomes

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Much of systems biology aims to predict the behavior of biological systems on the basis of the set of molecules involved and the complex network of interactions behind them. The last decade have seen the emergence of high-throughput proteomic initiatives for the identification of new protein interactions and macromolecular complexes that has led to the production of large interactome maps for several model organisms. The information contained in these interaction networks is mainly of binary nature, indicating whether one protein interacts with another. However, to fully understand how a complex system like the cell works we need to know the details of how these interactions are performed. Structural data for single proteins and complexes can unveil these details. It is therefore of paramount importance to combine these two sources of information to gain deeper insights on how biological processes are carried out inside the cell. In this talk, I will present our analyses on how interactomes take advantage of protein structural disorder to change and adapt through the course of evolution. In addition, I will also present a resource for the structural annotation of interaction networks that allows to easily collect and analyze all available structural knowledge on top of large interactomes.

P20-164
Pathogenic mutations impair nucleophosmin recognition of G-quadruplex forming DNA

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Nucleophosmin (NPM) is a nucleolar protein performing several functions that impact cell growth, among them, it assists the assembly and export of ribosomes, controls centrosome duplication and regulates the stability of tumor suppressors such as p53 and Arf. NPM is a multi-domain protein, composed of a pentameric compact core, long, flexible linkers, and small globular domains at the tips of each of its subunits. Besides binding a variety of proteins, NPM is able to interact with nucleic acids, which is probably related to its role in ribosome maturation. Although NPM is enriched in the nucleolus, it continuously shuttles into and out of the nucleus. Given the influence of NPM activity on cell proliferation and apoptosis, its dysregulation is linked to several human cancers. In particular, NPM gene is the most frequently mutated in adult acute myeloid leukaemia (AML), correlating with the aberrant localization of the protein in the cytoplasm, instead of the nucleolus. AML-linked mutations entail two features that jointly cause dislocation of the protein: (i) they generate a new export signal and (ii) prevent NPM C-terminal domain from folding, so that nucleolar retention fails. NPM anchorage to the nucleolus in physiological conditions may depend on its binding to nucleic acids, and AML-related unfolding of its C-terminal domain could alter this recognition. NPM has been recently described to preferentially associate with guanine-rich DNA sequences, which adopt characteristic structures known as 'G-quadruplexes'. We have approached the interaction of NPM with G-rich oligonucleotides, exploring the conformational requirements for the recognition. We have found that AML-associated mutations significantly hinder DNA binding ability in NPM.

P20-165
Structural and biochemical studies on multifunctional *Bacillus cereus* MCM complex

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DNA replication is strictly regulated through a sequenceof steps that involve many macromolecular protein complexes. One of them is thereplicative helicase, which is required for initiation and elongation phases. AMCM helicase found as a prophage in the genome of *Bacillus cereus*is fused with a primase domainconstituting an integrative arrangement of two essential activities forreplication. We have isolated this helicase–primase complex (BcMCM) showing thatit can bind DNA and displays not only helicase and primase but also DNApolymerase activity. Using single-particle electron microscopy and 3Dreconstruction, we obtained structures of BcMCM using ATPγS or ADP in theabsence and presence of DNA. The complex depicts the typical hexameric ringshape. The dissection of the unwinding mechanism using site-directed mutagenesisin the Walker A, Walker B, arginine finger and the helicase channels, suggeststhat the BcMCM complex unwinds DNA following the extrusion model similarly tothe E1 helicase from papillomavirus.

P20-166
Structural studies of the p22HBP/SOUL family of heme-binding proteins

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In 1998, p22HBP, a 22 kDa protein, was first purified from mouse liver cell extracts and characterized as a cytosolic, heme-binding protein by Taketani et al, though its functional role in the cell remains unknown. In 2006, the first structure of a protein from the SOUL/HBP family, mHBP, using NMR (Dias et al 2006). The mHBP structure presented a novel fold in eukaryotes and dissociation constants (K_d) for the mHBP-hemin and mHBP-PPIX complexes by fluorescence quenching (FQ) were found to be in the low nanomolar range and chemical shift perturbations arising from the addition of hemin and protoporphyrin IX (PPIX) were mapped to the mHBP structure allowing the interaction site to be determined. A subsequent molecular modelling study was performed, confirming the mHBP heme binding site and identifying some key charged residues at the edge of the binding pocket (Micaelo et al 2010).

In order to probe key interactions at the binding site mHBP mutants have been prepared by site-specific mutagenesis and NMR and FQ have been used to probe the resulting structural perturbations. The results indicate that chemical shift deviations were found for residues located near the mutation sites and all mutants were found to be structurally similar to wild type mHBP as expected. The resulting dissociation constants, obtained by FQ, show that the modification of these residues does not affect significantly binding.

Human SOUL (hSOUL) is a 23 kDa heme-binding protein initially identified in the retina and pineal gland of chicken and recent studies suggest that hSOUL is involved in necrotic cell death by inducing mitochondrial membrane permeability (Szigeti et al 2010). X-ray diffraction data were collected to 3.5 Å resolution and a model of the 3D structure of hSOUL, obtained by molecular replacement, using the NMR structure solution of mHBP indicated a conserved fold when compared to the mHBP structure (2YC9) (Freire et al 2009). A recent X-ray structure confirmed our structural results and indicated that hSOUL does not bind heme (Ambrosi et al 2011). Triple resonance spectra have been acquired for 13C/15N and 2H/13C/15N labelled hSOUL samples and the backbone assignment of hSOUL allowed us to follow chemical shift alterations upon heme titration and relaxation data has been used to identify dynamic alterations upon heme titration.

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P20-167
Functional analysis of site-directed mutants from the active site of higher plant glutamine synthetase

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We have examined by site-directed mutagenesis the functionality of highly conserved residues from the active site of glutamine synthetase α (α-GS) from *Phaseolus vulgaris*. Two different types of residues were analyzed: (i) residues involved in the reactivity of the enzyme with ammonium (Asp-57, Glu-297); (ii) residues involved in the reactivity with ATP and/or metal cofactors (Arg-316, His-249). Results will be shown on the main kinetic parameters determined for wild-type and mutant enzymes as well as fluorescence and isothermal calorimetry studies of the binding of α-GS towards different substrates. In addition, we will show the results of the analysis of different lines of transgenic plants of *Lotus japonicus* that were transformed with E297A and H249N mutant versions of GS. Acknowledgements: Authors wish to thank financial support given by Junta de Andalucía (Spain) P.O. FEDER 2007-2013 (Project P07-CVI-3026) and BIO-163.

P20m-168
A structural and photopysical study on lysozyme aggregation in physiological conditions induced by negatively charged liposomes

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Hen egg-white lysozyme (HEWL) has been extensively used as a model to investigate protein folding and aggregation, protein adsorption at interfaces and membrane. Evidence indicated that HEWL aggregates into amyloid-like prefibrillae and fibrillae when incubated at pH 2 and 60°C for some days but this transition from native to amyloid-like structure is also triggered by lipid membranes in physiological conditions. In the present study we sought to obtain further and more detailed information on the mechanisms of interaction of HEWL with lipid vesicles in terms of surface-induced protein conformational variation and subsequent aggregation. In particular, we investigated the variations of the secondary structure of HEWL in presence of liposomes with different surface charge density resulting from various molar ratios of zwitterionic POPC and negatively charged POPS and POPG. The fusion of negatively charged liposomes by interaction with the HEWL was observed trought Dinamic Light Scattering and as the negative surface charge of liposomes increased, an alteration of native HEWL secondary structure was detected by Circular Dichroism analysis. Besides, an accelerated aggregation kinetics was observed by ThT binding experiments. The effect of HEWL on liposomal membrane fluidity and on vesicle fusion was investigated by fluorescence polarization of 1,6-diphenyl-1,3,5-exatriene, a decrease of liposome membrane fluidity occurred upon binding to HEWL. We also observed a reduced HEWL enzymatic activity in presence of lipo-

somes. Electron Microscopy revealed amyloid-like fibrils structures that showed to be cytotoxic as confirmed by MTT assays. Eventually, Confocal Microscopy experiments performed on Giant Unilamellar Vesicles incubated with HEWL further validated protein/liposome interaction and induced vesicle fusion. POPG or POPS liposomes incubated with HEWL induce the formation of amyloid-like structures. Elucidation of the intrinsic kinetic interplay between amyloidogenesis and membranes provides a challenge that needs to be addressed to completely ascertain the role of membranes in amyloid disease pathology.

P20-169
Production of bovine transforming growth factor beta 1 following stimulation with lipopolysaccharide

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Research in agricultural sciences requires extensive knowledge and it must be comprehensive [1]. Bovine mastitis is the most costly disease of dairy cattle and therefore it is intensively investigated [2,3,5]. Very important part of research in mastitis is investigation of inflammatory cytokines [4]. Transforming growth factor beta (TGFβ) is a cytokine which exists in five isoforms. TGFβ1 is produced in very high levels by platelets. Other types of cells which produce this cytokine are macrophages, lymphocytes, endothelial cells, fibroblasts, neurons etc. Lipopolysaccharide (LPS) has an ability to induce inflammatory response of bovine mammary gland. The aim of this study was to evaluate concentration of TGFβ1 in course of *in vitro* cultivation of bovine mammary gland leukocytes with LPS. Heifers were used as mammary gland cell donors for *in vitro* studies. Intact leukocytes from the mammary glands were harvested following the phosphate buffered saline intramammary injection. TGFβ1 concentration was quantified by ELISA. LPS stimulated TGFβ1 production in bovine leukocytes. The concentration of TGFβ1 was gradually increasing during four-hour incubation. These preliminary data need next exploration to reveal detail effect of LPS on production of cytokines in connection with inflammatory process.

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P20-170
Ligand recognition by TEM-1 beta-lactamase allosteric mutant

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Beta-lactam antibiotics are the most produced and used antibacterial drugs in the world. Unfortunately, beta-lactamase mediated antibiotic resistance has become a major health problem. Beta-lactamase inhibition, used in combination therapies with antibiotics, is a powerful strategy in the fight against infectious diseases. *Streptomyces clavuligerus* produces beta-lactamase inhibitor protein (BLIP), which inhibits a wide range of class A beta-lactamases. Understanding the inhibitor recognition mechanism of beta-lactamases may help to combat against beta-lactamase mediated antibiotic resistance. Most inhibitors bind to the active site and inhibit beta-lactamase in a competitive manner. Examination of several class A beta-lactamases using sequence and structure homology revealed the presence of a highly conserved residue, Trp229 near the allosteric inhibitor binding site, which was previously identified in class A beta-lactamases TEM-1 and SHV-1. In this study, experimental and computational mutagenesis of Trp229 to Ala was performed in order to investigate the effect of mutation to the binding affinity towards BLIP. Molecular dynamics simulations were carried out in unbound and BLIP bound forms of both wild type and W229A mutant TEM-1 beta-lactamase. The simulation trajectories were analyzed to obtain information about the changes in mobility and energetics upon mutation. The trajectory analysis shows that the mutant TEM-1 beta-lactamase has higher H10 fluctuations in the presence of BLIP, higher affinity to BLIP and higher cross-correlations with BLIP. The effect of this mutation was also tested experimentally using kinetic experiments on the W229A mutant of TEM-1 beta-lactamase. The experimental results were compared with the computational findings in order to elucidate the communication between the active site and the allosteric site of TEM-1 beta-lactamase. Funding by TUBITAK (109M229) is gratefully acknowledged.

P20-171
Implications of the nucleocytosolic localization of soluble pyrophosphatases in yeast and human cells

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Pyrophosphate (PPi) removal is essential for most central biosynthetic reactions to proceed in the appropriate direction. Recently we have determined the cellular consequences of a deficiency in yeast Ipp1p (a Family I inorganic pyrophosphatase, sPPase). This essential enzyme shows a nucleocytosolic localization. However the cellular and molecular significance of this localization is not known. We have engineered yeast strains expressing this sPPase in the nucleus or in the cytosol only by merging the *IPP1* ORF to a basic Nuclear Localization Signal or to a leucine-rich Nuclear Exclusion Signal, respectively. Protein and activity levels were observed to be significantly different between these two scenarios: both were much smaller when Ipp1p was confined into the nuclear compartment. This was accompanied by slower growth rates and reduced chronological life-span. We are currently working on the molecular mechanisms involved. Although

two Families (I and II) of non-homologous sPPases have been reported, they show a remarkable conservation in the spatial conformation of the active site and in the catalytic mechanism. To compare their functional efficiency *in vivo*, we have expressed a number of sPPases from different sources (bacteria and plants) and native subcellular localizations (cytosol, plastids, mitochondria), both in *Saccharomyces cerevisiae* and human cell lines. Different expression levels and subcellular localizations were observed for each sPPase in the heterologous systems. On the whole, a correlation between expression levels, cell growth and ability to functionally complement a deficiency in the autologous sPPase was observed. Supported by grant P07-CVI-3082 from Andalusian Administration (FEDER).

P20-172
Solution structure of a mutant of the triheme cytochrome PpcA from *Geobacter* sulfurreducens sheds light on the role of the conserved aromatic residue F15

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Extracellular electron transfer is one of the physiological hallmarks of *Geobacter* cells [1]. The genome of most *Geobacter* species encodes for more than 100 *c*-type cytochromes which are, in general, poorly conserved. The exception is the PpcA family composed by triheme *c*-type periplasmic cytochromes, which are the most abundant in these bacteria [2]. The functional characterization of PpcA showed that it has the adequate properties to couple e^-/H^+ transfer, a fundamental step for ATP synthesis [3]. The detailed thermodynamic characterization of a PpcA mutant, in which the strictly conserved residue phenylalanine 15 was replaced by leucine (PpcAF15L), showed that the global redox network of cooperativities among the heme groups is altered preventing the mutant to perform a concerted e^-/H^+ transfer [4]. In this work, we produced ^{15}N-labelled PpcAF15L and used NMR spectroscopy to determine its solution structure in the fully reduced state and to screen the pH-dependent conformational changes. The structure obtained is well defined, with an average pairwise root-mean-square deviation of 0.36 Å for the backbone atoms and 1.14 Å for all heavy atoms. The comparison between the solution structures of PpcAF15L and wild-type proteins allowed mapping the structural origin for the disruption of the concerted e^-/H^+ transfer.

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P20-173
Functional analysis of the role of selected ribosomal proteins in the biogenesis of large ribosomal subunits from the yeast *Saccharomyces cerevisiae*

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Ribosome synthesis involves the concomitance of pre-rRNA processing and ribosomal protein assembly. In eukaryotes, this is a complex process that requires the participation of specific sequences and structures within the pre-rRNAs, at least 200 *trans*-acting factors and the ribosomal proteins (r-proteins). There is little information on the function of individual 60S r-proteins in ribosome synthesis. Herein, we present data on the contribution of different evolutionarily conserved r-proteins in yeast ribosome biogenesis.

First, we will show the characterisation of dominant negative mutants of L3 r-protein, which we believe is assembled by selected RNA helicases *in vivo*.

Second, we are currently assessing the function of L14 and L16 r-proteins, which are intimate interacting in the mature 60S r-subunit. We are especially interesting in understand the interdependence of these two proteins during assembly.

Finally, we will discuss on the role of other r-proteins such as L40, which is naturally fused to ubiquitin. Our data clearly indicate that L40 is a very late/cytoplasmic assembly r-protein involved in translation elongation.

We conclude that r-proteins play an active role on ribosome biogenesis and are not mere passive actors in this process.

P20-174
Heterological expression and purification of Chd64 from Drosophila melanogaster

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Juvenile hormone (JH) and ecdysteroids regulate a wide variety of developmental and physiological processes in insects. Two proteins, calponin- like protein Chd64 and immunophilin FKBP39, that bind to DmJHRE1 (Drosophila melanogaster JH response element) were previously identified. It is was shown that FKBP39 and Chd64 can interact with each other and with ecdysteroid receptor, utraspiracle nuclear receptor and methoprene- tolerant protein. This suggests that Chd64 and FKBP39 probably play important roles in cross- talk between JH and ecdysteroids. To facilitate the exploration of the interrelationship between function and molecular properties of Chd64, we developed and optimized a protocol for the efficient expression and purification of this protein. In order to express the recombinant protein DmChd64 in *Escherichia coli*, recombinant plasmid vectors pQE80L-XH, pQE80L-SX and pQE80L-SXH containing cDNA coding for the target protein in fusion with: His-tag (XH), Strep-tag (SX) and both of those tags (SXH) were prepared. We confirmed that all expressed recombinant proteins remained in the soluble fraction of *E. coli* proteins and that those fused with the His- tag and Strep- tag were able to be bound by the TALON® and StrepTactin® resin. Finally, we elaborated a two-step purification procedure for the homogenous Chd64 using affinity chromatography (TALON® resin and StrepTrap® column). The molecular mass value (23321.76 ± 2 Da) was determined using electrospray ionization (ESI) mass spectrometry and is compatible with theoreti-

cal value (23453.3 Da). This enables further molecular and structural characterization of Chd64 using a diverse array of biochemical and biophysical methods.

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P20-175
Allosteric communication between subunits of the dimeric phosphofructokinase-2 of *E. coli* analyzed by hybrid enzymes and molecular simulations

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The activity of phosphofructokinase-2 (Pfk-2) is downregulated by allosteric MgATP binding, avoiding a futile cycle of ATP hydrolysis under gluconeogenic growth conditions. Kinetic studies show that the inhibition is associated with a change in the saturation for the substrate fructose-6-P from a hyperbolic to a sigmoidal behaviour, with a decrease in its apparent affinity. This change suggests allosteric communication between the fructose-6-P binding sites of the monomers according to specific allosteric communication models. The catalytic and allosteric sites in each monomer of the Pfk-2 structure, involve residues from both subunits, indicating that the dimeric structure is closely related with the function and regulation of the activity of the enzyme. In order to determine the allosteric inhibition mechanism of Pfk-2, subunit hybrid enzymes of Pfk-2 mutants were constructed and characterized kinetically. The hybrids dimers that contain only one intact site for fructose-P and one catalytic site in the same subunit, show hyperbolic saturation curves for fructose-6-P and is inhibited by MgATP, since the allosteric sites for the nucleotide are intact in both subunits. However, the hybrid dimer that contains only one intact allosteric site and a catalytic site in same subunit shows MgATP inhibition and sigmoidal response for fructose-6-P. Molecular dynamic simulations in the presence of the allosteric and catalytic ATP, show main chain correlated movements between the fructose-6-P and allosteric binding sites, in agreement with the kinetic experiments. These results indicate that the sigmoidal saturation behaviour for fructose-6-P is due to communication between the fructose-6-P sites. Also, the allosteric communication between subunits would not play an important role in the MgATP inhibition. (Supported by *Fondecyt* 1090336, Chile).

P20r-176
Experimental paleogenetics and paleobiochemistry to study the evolutionary history of substrate specificity in archaeal ADP-dependent sugar kinases family

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In several archaea of the *Euryarchaeota* group the glycolytic flux presents ADP-dependent glucokinase and phosphofructokinase (PFK) activities. These enzymes are homologous and belong to the ADP-dependent sugar kinases family of the ribokinase superfamily. Interestingly, the enzyme from *Methanocaldococcus jannaschii* is capable to use both sugars, and has been proposed to be an ancestral form of the family. However, recent studies have shown that the ancestral activity in the group is glucokinase and that the bi-functional enzyme belongs to the phosphofructokinase subfamily. Since experimental paleogenetics and paleobiochemistry provide an opportunity to investigate in the laboratory the molecular history of modern organisms and in order to understand the evolutionary history of this family we reconstructed the phylogenetic tree for the archaeal branch using the Bayesian method. Also, we inferred, synthesized and expressed the gene for the last common ancestor of phosphofructokinases from *Thermococcales* and *Methanococcales* groups (ancMT) and the PFK ancestor of the *Thermococcales* group (ancT). From this data we inferred the evolutionary history of substrate specificity and compared it with the experimental evidence obtained for our resurrected ancestral enzymes. We found that the ancMT ancestor is capable to use glucose and fructose-6-P as substrate, while the ancT ancestor is specific for fructose-6-P and that of the Methanococcales group should be bi-functional. The results support the idea that the *M. jannaschii* enzyme is not an ancestral form of the family. Also indicates that enzymes from the *Methanococcales* organisms conserved the ancestral trait of bi-functionality whereas the specificity for fructose-6-P is an acquired trait in enzymes of the *Thermococcales* group (Fondecyt 1110137).

P20-177
Point mutations preventing post-translational modifications abolish intracellular localization of recombinant Rab7b in the model eukaryote

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Post-translational modifications may affect the function and properties of the proteins. Recombinant Rab7b and its mutagenized variants were used to elucidate factors determining localization of this protein that acquired a new function in unicellular eukaryote *Paramecium octaurelia*. In the recombinant His-Rab7b constructs Ser140 or Thr200, respectively, were replaced with alanine not undergoing post-translational modifications. The bioinformatic analysis showed that Ser 140 and Thr 200 in Rab7b are the two unique putative glycosylation and phosphorylation sites as compared to the product of the ohnologous gene Rab7a exhibiting both the different localization and function in the late phagosomal trafficking. Interestingly, besides the divergent C-termini only other difference between the amino acid sequences of these two proteins is the substitution of alanine at position 140 in Rab7a with serine in Rab7b. Incorporation of His-Rab7b or its variants was studied upon *in vivo* electroporation under the same conditions concerning the ratio between protein amount and the number of cells in each sample. After four hours of recovery cells were fixed and processed for double fluorescence immunodetection in confocal microscopy using two sets of antibodies enabling the two-colored detection. Only His-Rab7b localized correctly in the cell as the endogenous protein. Two other recombinant Rab7b mutagenized species were abnormally incorporated in the cells indicating that post-translational modifications may affect protein targeting and contribute to neofunctionalization of the products of the duplicated genes.

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P20-178
Mechanism of L-PK regulation by phosphorylation

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Pyruvate kinase (PK, ATP:pyruvate 2-O-phosphotransferase, EC 2.7.1.40) catalyzes final step of glycolysis transferring phosphoryl

group of phosphoenolpyruvate to ADP producing pyruvate and ATP. In mammals there are four PK isoenzymes: M1, M2, R and L. Only the activity of L-type pyruvate kinase, found in liver, can be regulated by phosphorylation on serine 12 residue of small N-terminal domain: MEGPAGYLRR^{10}AS^{12}VAQLTQEL^{20}GTAFF. As a result of phosphorylation of this peptide fragment affinity of L-PK to phosphoenolpyruvate decreased. Moreover, the phosphorylation reaction was found to act as a switch of cooperativity of the enzyme in reaction with this substrate. To investigate the mechanism of functioning of this cooperativity switch point mutations were introduced into the phosphorylatable area in positions 9, 10 and 13. It was found that some of these mutations (R9E, R9Q, R9K, R10Q) influenced on L-PK affinity for PEP, while had no effect on the enzyme affinity against its second substrate ADP. Computer modeling of docking of ligands, including fragments of the phosphorylatable N-terminus, to the enzyme molecule was used to characterize the binding sites of substrates and the regulatory peptide fragments that was necessary to develop the general pictute of the regulation mechanism.

P20-179
Elucidation of the two-electron transfer process in fumarate reductase from Shewanella

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In the absence of molecular oxygen, some bacteria can use a variety of terminal electron acceptors for respiration, including nitrate, sulfate, insoluble Mn(IV) and Fe(III) oxides and fumarate. Fumarate respiration is one of the most widespread modes of anaerobic respiration, probably because fumarate can be formed from carbohydrates and proteins. The fumarate reductase of *Shewanella*, is the flavocytochrome Fcc_3, a soluble tetraheme cytochrome of 64 kDa that performs unidireccional fumarate reduction with a FAD group co-factor in the active site. X-ray crystallography showed that this protein folds in three domains: the N-terminal domain that contains four *c*-type cytochromes, the C-terminal domain with a non-covalently bound FAD-group and a clamp domain that is involved in controlling access of the substrate to the active site of the enzyme. The four hemes are positioned in a quasi-linear arrangement that allow electrons to flow from the redox partner to the buried FAD group located at the catalytic site, for the reduction of fumarate. The thermodynamic properties of Fcc_3 showed that the individual reduction potentials of the hemes are organized in a way that allows a sequential transfer of two electrons to the FAD group. However little is known about the kinetic of electron transfer. Up to date, studies were only performed at a macroscopic level, showing that the electron transfer to the FAD group is slower than within the heme domain. The factors that control the electron transfer process in this multi-centre redox protein and that regulate the two-electron transfer that is essential for the catalytic process remains to be elucidated. Kinetic studies will reveal the reduction and oxidation process performed by Fcc_3 and the contribution of each redox centre will be determined using a kinetic model that allows the discrimination of each individual heme. This information is essential to reveal how a chain of single electron redox co-factors such as hemes is capable of loading a two-electron active site such as the FAD, which in flavocytochrome c_3 is known not to exist in the semiquinone state.

P20-180
Solution structure and biochemical studies on C-terminal domain of *Vibrio vulnificus* extracellular metalloprotease

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Vibrio extracellular metalloprotease (vEP) secreted by *Vibrio vulnificus* ATCC29307 exhibits various proteolytic function such as prothrombin activation and fibrinolytic activities. Premature form of vEP is composed of an N-terminal propeptide region (nPP), a catalytic and C-terminal domains. The nPP region is autocleaved for the matured metalloprotease activity. However, there has been no direct evidence that C-terminal domain can modulate vEP's substrate specificity. To understand how C-terminal domain of vEP modulate its proteolytic activities, NMR studies on C-terminal domain of vEP $_{(510\text{-}510)}$ were performed by heteronuclear NMR spectroscopy. The solution structure revealed that $vEP_{510\text{-}610}$ forms a central β-barrel composed of seven anti-parallel β-strands with two metal ion binding sites. Data from hydrogen/deuterium exchange experiments together with ^{1}H-^{15}N heteronuclear NOE (XNOE) suggest that $vEP_{510\text{-}610}$ has a highly compact structure. Through DALI server analysis based on solution structure, $vEP_{510\text{-}610}$ shares a structural similarity with collagen binding domain of collagenase although it has a low sequence homology. Our structure proposes that C-terminal domain of vEP could interact with collagen during the modulating a process of proteolytic activities in the infect pathway systematically. This work was supported by a NRF grant funded by the MEST (J. S. Lee and W. Lee, 20110027675).

P20-181
Structural and dynamical insights on HLA-DRB1: peptide complexes which confer resistance and susceptibility to multiple sclerosis in sardinia: a molecular dynamics simulation study

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Multiple Sclerosis is an autoimmune disease linked to inflammatory and degenerative process in the central nervous system. Human Leukocyte Antigen (HLA) class II system has been identified as the main genetic determinant regions linked to MS [1]. Recent genetic studies [2], have identified and associated five HLA-DRB1 alleles to Multiple Sclerosis (MS) in Sardinia. The basis of adaptive immune response has been associated with recognition of peptides bound to specific membrane glycoprotein, the Major Histocompatibility Complex (MHC) by T-cell antigen receptors (TCR) [3]. Antigen/Peptide presentation by MHC class II is critical component of the adaptive immune response to foreign pathogens. The availability of high resolution x-ray structures of the complexes in some cases have provided structural insights for antigen presentation. Our research is focused on investigation of MHC class II peptide interaction with an emphasis on identifying structural and dynamical differences between the predisposing and protective DRB1 alleles complexed with both self: Myelin Basic protein (MBP) and non-self Epstein Barr Virus (EBV) peptide alternatively at a microscopic level. Our detailed analysis confirm that a functional relation between MS predisposing genetic background and antigen presentation can be investigated by MD simulations.

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P20-182
Structural and functional studies of two component system SCO3062/3063 of *Streptomyces coelicolor*

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The two component signal transduction system (TCS) is widely distributed in most organisms except for animal cells and plays an important role in response to environmental stimuli of cells. Since TCS is involved in virulence and anti-bacterial resistance, bacterial TCS is an attractive target for developing anti-bacterial inhibitors. Here, we characterized the TCS of histidine kinase SCO3062 and its response regulator SCO3063 of *Streptomyces coelicolor*. The SCO3062/3063 TCS was identified by auto-phosphorylation of the cytoplasmic domain of SCO3062 and phospho-transfer to SCO3063 from SCO3062. In addition, structural analysis of the sensor domain of SCO3062 using NMR and CD (circular dichroism) revealed a dramatic conformational change in different pHs. While the protein was well-folded under acidic condition (pH < 5.0), the protein structure was destabilized under basic condition (pH > 7.0). The conformational change was reversible in the pH range of 2.5–10. This is unusual phenomenon since protein structure is unstable at low pHs. From CD data, the transition point between two forms was around pH 6.0. Interestingly, histidine residue, which can induce conformational changes of protein structure around pH 6.0 by protonation of histidine side chain, is absent in the sensor domain. We found that the Glu83Ala mutant destabilized the structure of sensor domain at both high and low pHs. On the other hand, the Glu83Gln mutant, which prevents protonation of side chain at low pH but can maintain hydrogen bonds with other residues, shifted the transition point up to around pH 7.0. On the basis of these data, we suppose that the pKa of Glu83 side chain is abnormally high and the protonation of side chain at low pH stabilizes the structure of sensor domain through hydrogen bonds between the protonated side chain and other residues. However, the conformational change may be induced not only by Glu83 but also by other acidic residues since the conformational change is still occurred in Glu83Gln mutant. These data suggest that the TCS of SCO3062/3063 may sense acidic condition of extracellular environment and regulate internal pH of *Streptomyces coelicolor*.

P20-183
DNA binding properties of 14-3-3 protein family

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The 14-3-3 proteins are a highly conserved protein family which is essential for basic cellular processes from yeast to mammals. They belong to the first discovered signal molecules that are specifically bound to the motives containing phosphoserine or phosphothreonine and the role of 14-3-3 proteins are realized by proteins and DNA interactions. Nine isoforms (α, β, γ, δ, ε, η, σ, τ and ε), encoded by seven distinct genes, have been identified in mammals. It was shown that 14-3-3 proteins are involved in eukaryotic DNA replication via binding to the cruciform DNA and that some isoforms have a strong preference for DNA in cruciform conformation. In our study we analyze DNA-binding properties of six different bacterially expressed 14-3-3 protein isoforms. We compare 14-3-3/DNA binding properties to linear and supercoiled DNA of different plasmids with and without inverted repeats capable to form cruciform structure. We demonstrated that 14-3-3 proteins bind strongly to long DNA targets, as evidenced the appearance of blurry, retarded DNA bands on agarose gels. Isoforms β, γ, δ and σ bind to superhelical plasmid with extruded cruciform structure. The isoform γ bound to plasmid DNA at lowest concentration. Competition experiments with linear and supercoiled DNA on magnetic beads show very strong preference for supercoiled DNA. We suppose that the capability of 14-3-3 proteins to bind directly to supercoiled DNA could play an important role their regulatory functions.

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P20-184
Molecular mechanisms of cytochromes involved in extracellular electron transfer

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In recent years, new methods of clean and environmentally friendly energy production, such as MFCs, have been the focus of intense research efforts. The natural habitats of bacteria thriving in MFCs are usually marine and freshwater sediments. These bacteria typically have a remarkably versatile bioenergetic metabolism, often associated with formation of biofilms and the exchange of electrons with extracellular solids.

Several organisms have been the focus of research because of their capability to donate electrons to an anode, such as Geobacter and Shewanella, or their capability of extracting electrons from a cathode, such as Rhodopseudomonas and Rhodobacter.

The use of extracellular electron acceptors and donors requires a novel arrangement of the respiratory chains, to couple electron transfer to and from the outside of the cell to the production of ATP at the inner membrane. A hallmark of organisms performing extracellular electron transfer is the presence of many redox proteins including various cytochromes.

One of the most studied species that perform extracellular respiration is *Shewanella oneidensis* MR-1, whose genome contains numerous c-type cytochromes. Several of which have been implicated in the bioenergetic metabolism that uses soluble and insoluble metal compounds as terminal electron acceptors.

The detailed functional characterization of the small tetraheme cytochrome (STC or CctA) from *S. oneidensis* MR-1 and *S. frigidimarina* showed that there is functional specificity and directional electron transfer, despite the absence of specific interactions with physiological partners. This reveals that individual protein characteristics can be important in determining the electron flow direction.

This research aims to identify the amino acids that are important in the electron transfer mechanism between STC and metal oxides, and interactions with physiological redox partners. For this purpose, 15 mutants of surface residues were created and

their detailed thermodynamic and kinetic characterization will be presented.

P20-185
In silico study of 3D structure of human aryl hydrocarbon receptor and of its role as chemosensor molecule

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Humans are constantly exposed to an enormous number of chemical molecules present in his environment. Depending on their chemical properties, 'xenobiotics' that enter the cell can affect cellular function by either non-selective binding to cellular macromolecules or by interference with cellular receptors, in particular nuclear receptors that function as regulators of transcription. One of these intracellular chemosensor molecules is the aryl hydrocarbon receptor (AhR), a transcription factor of the basic helix_ loop_helix/Per-ARNT-Sim (bHLH/PAS) family that is known to mediate the biochemical and toxic effects of dioxins, polyaromatic hydrocarbons and related compounds. In our study computational methods have been applied in order to define structure of human AhR- Ligand Binding Domain (hAhR-LBD), including PASB and PAC regions, and to characterize interaction of this protein domain with different ligands. The model of hAhR-LBD obtained by homology modelling was used to docking simulations with some molecules among the most important and potent AhR ligands. A future study aim is to perform molecular dynamics simulations, in order to describe interaction mechanisms (and maybe competitive behaviour) between hAhR-LBD and two ligands for time. For each one of the molecule tested, a specific 'binding fingerprint' was traced. In particular for dioxin and other toxic molecules tested, these data allow to identify the most important residues in the domain involved in binding and that could be used as a 'peptide sensor'. Finally we are interested in (i) a structural study and analysis of the other protein domains, in particular of PASA domain and DNA binding domain; (ii) characterization of AhR interaction with the other members of AhR complex.

P20-186
Determination of the biochemical and structural properties of CUP adhesins from *Peudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen responsible for ~10% of worldwide nosocomial infections. It is responsible for chronic or acute bronchopulmonary infections in immunocompromised patients that are the major cause of mortality in cystic fibrosis (CF) patients. The infections are almost impossible to eradicate by classical antibiotic treatments due to the development of multiresistant strains and adaptive changes to a biofilm phenotype. The host recognition and adhesion are mediated through lectin-carbohydrate interactions, during the early steps of infection. *P. aeruginosa* virulence is in part related to the production of an arsenal of lectins which are either: soluble, flagellar or fimbrial. These lectins are involved either in oligosaccharide-mediated recognition and adhesion or/and in biofilm formation or/and maturation. Some fimbrial lectins also called adhesins are presented at the distal end of adhesive pili secreted by the conserved Chaperone Usher Pathway (CUP). Recently, at least five gene clusters: encoding CUP pili, cupA-E, have been identified in the *P. aeruginosa* genomes. Our investigations are focused on the CupB6 adhesin through a multidisciplinary study from the gene to the structure in order to identify its functional role and the molecular basis of its sugar specificity. One strategy to obtain soluble recombinant CupB6 adhesin is to coexpress it together with its chaperone CupB4. The chaperone stabilizes the protein and prevents it from premature polymerisation. To face the difficulties encountered to get crystals, we've carried out thermal shift assay on the complex in order to determine more suitable buffer conditions to stabilize it. By this approach, crystals have been recently obtained but they need to be further optimized to be suitable for diffraction studies. In parallel, we used SAXS in order to get the envelope of this complex. At the moment, the identification of CupB6 natural ligand is under way using glycan array, surface plasmon resonance (SPR) and isothermal microcalorimetry (ITC) technics.

P20-187
Unraveling electrostatic interactions in the active site of photoactive yellow proteins

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The ability to avoid blue light radiation is very crucial for life sustainability of bacteria. Photoactive yellow protein (PYP) shows a ultra violet (UV) absorption spectrum, which matches with the wavelength dependence of the escape of the bacterium from potentially harmful blue-light radiation. Its response to the blue light is associated to the changes of the optical property of chromophore *para*-Coumaric acid (*p*CA) in the active site of PYP and display strong pH dependence in photocycle kinetic (1). Due to this observation, PYP has become an important example of rich model system for the study of Per-arnt-sim (PAS) domain signaling and a soluble bacterial light sensor. However, the mechanism of pH dependence in the photocycle kinetics of PYP is still in dispute, in which some experimental and theoretical studies are contradictive. Therefore, a comprehensive *p*Ka determination is crucial in order to clarify the mechanism. In this study, we successfully determine all individual protonation states of titrable groups in the active site of PYP based on heteronuclear NMR chemical shift. To achieve that goal, we utilize a novel NMR approach to sequence-specifically follow all tyrosine side chain protonation states (2). At the same time, we also develop a simple and cheap labeling strategy to determine the *p*Ka of arginine in PYP. These findings provide the insight into the electrostatic interaction in the active site of PYP and resolve the ambiguity of the pH-dependence in the photocycle kinetic.

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P20-188
Lim-domain mediated autoinhibition of a mammalian agmatinase-like protein

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Agmatine, decarboxylated arginine, has been associated to several processes in mammals, including neurotransmitter, anticonvulsant, antineurotoxic and antidepressant actions in the brain. Agmatine levels would be, certainly, regulated by synthesis by arginine decarboxylase and hydrolysis by some agmatinase. There has been, however, difficulties in detecting an active brain agmatinase, thus difficulting their enzymatic characterization. We recently cloned and immunohistochemically detected a rat brain agmatinase-like protein (ALP), whose amino acid sequence greatly differs from other agmatinases and exhibits a C- terminal LIM-domain, for this domain we have builded a structural model. The protein was detected in the hypothalamic region, hippocampal astrocytes and neurons. Since truncated species, lacking the LIM-type domain exhibits a 10-fold increased k_{cat}, and a three-fold decreased K_m value for agmatine, our proposal has been that the domain functions as an autoinhibitory entity in ALP. To evaluate this suggestion, we have now cloned and expressed the isolated domain. The isolated purified LIM-domain contained 2 Zn^{2+} ions, which were are absent from the truncated enzyme, and resulted to be inhibitory to truncated species, but not to the wild-type enzyme. The inhibition of the truncated species was competitive, with a K_i value of 0.2 μM. From these results, which reinforce our postulated autoinhibitory role, our suggestion is that the LIM-domain portion of ALP interacts with a yet unidentified brian protein and, as a consequence, the inhibition of the enzyme is released. The LIM-domain would play, therefore, a critical role in the regulation of agmatine concentrations in the brain. Grant Fondecyt 1120663.

P20-189
Bacterioferritin from *Desulfovibrio vulgaris* Hildenborough is a functional DPS-like enzyme

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Iron is an essential transition metal for living organisms. However, it can be seen as a potential problem due to Fe(II) toxicity in the presence of O_2 or H_2O_2,and poor solubility of Fe(III). Reaction of Fe(II) with H_2O_2 via Fenton chemistry produce reactive oxygen species (ROS) such as hydroxyl radical or superoxide. Mechanisms used to protect cells against ROS include the accumulation of anti-oxidant species and expression of specific enzymes. Peroxidases, catalases or superoxide dismutases, belonging to the ROS detoxifying enzyme family, are generally present in aerobic organisms [1]. To minimize ROS production, and simultaneously deal with iron bioavailability, organisms utilize a family of proteins, the ferritin family, that are able to oxidize ferrous ions and store large amounts of iron in a ferric oxide mineral form, inside a hollow protein shell, formed by the assembly of 12 or 24 subunits of approximately 20 kDa [2]. A bacterioferritin coding gene was identified and isolated from the genome of *Desulfovibrio vulgaris* cells, and overexpressed in *E. coli*. *In vitro*, H_2O_2 oxidizes Fe(II) ions at much higher reaction rates than O_2. H_2O_2 oxidation of two ferrous ions was proven by Mössbauer spectroscopy. Based on the Mössbauer parameters of the intermediate species we propose that *Desulfovibrio vulgaris* bacterioferritin follows a mineralization mechanism similar to the one reported for vertebrates H-type ferritins. This bacterioferritin is also able to bind different DNA structures. Use of H_2O_2 as oxidant, combined with the DNA binding activity seems to indicate a Dps-like role for *Desulfovibrio vulgaris* bacterioferritin.

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P20-190
Self-association of the regulatory domain of human phenylalanine hydroxylase: contribution to the protein instability

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Human phenylalanine hydroxylase (hPAH, EC 1.14.16.1) is a non-heme iron enzyme that catalyzes the hydroxylation of L-phenylalanine (L-Phe) into L-tyrosine, in the presence of the cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4) and dioxygen. A deficient activity of hPAH leads to phenylketonuria (PKU; OMIM 261600) and related forms of hyperphenylalaninemia (HPA). PKU is often considered a protein misfolding disease with loss-of-function. Human PAH has a small margin of stability, and a high frequency of misfolded hPAH mutant proteins are associated with PKU/HPA, resulting in enzymes with a propensity to self-associate and to form higher-order oligomers (when overexpressed in prokaryotic systems) and to be rapidly degraded (when expressed in eukaryotic cells). This group of mutations does not directly affect the catalytic function, but impairs molecular motions involved in regulatory processes, substrate and cofactor binding and oligomerization assembly. Particularly the N-terminal regulatory domain (R-domain; containing a βαββαβ motif (ACT domain)) seems to play a crucial role in the instability and misfolding of the protein.

We have expressed and isolated the R-domain (residues 2-120) of WT-hPAH and the severe mutant form G46S and observed that not only the mutant domain self-associates but also the isolated WT R-domain. However, L-Phe inhibits in a stereospecific manner the self-association of the WT, but not of the mutant. The available data suggest that although present in the R-domain, the full-length hPAH has lost the second L-Phe binding site as a result of a conformational change in the R-domain and/or a steric hindrance due to interdomain interactions. The gain of regulatory properties and the loss of the ability to bind L-Phe may contribute to the observed instability of the R-domain (wild-type and mutants) and its involvement in protein misfolding.

P20-191
Structural studies on up-regulated gene 7 (URG7)

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Up-Regulated Gene 7 (Urg7) encodes a protein 99 amino acids long that presents a high homology with the protein product of

the pseudogene 2 (ABCC6Ψ2) of the ABCC6 gene [1]. Is known that URG7 contributes to the survival of HBV-infected hepatocytes during chronic infection. In particular, HBxAg activation of the proinflammatory NF-KB results in the up-regulation of URG7 that inhibits TNFα-mediated killing by inhibiting caspases 3 and 8 activity in the apoptotic pathway and by activating phosphoinositol 3-kinase and β-catenin [2]. In order to obtain information about the structure and function URG7 has been expressed in *E. coli* and purified by RP-HPLC. CD spectroscopy studies performed in the far UV (190-250 nm) have shown a heterogeneous population of α-helice and β -secondary structures. Moreover, URG7 has been expressed in *E. coli* with 15N isotope-labelling for further structural characterization by NMR spectroscopy.

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P20-192
Characterisation of PDIp- a pancreas-specific homolog of PDI

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The protein disulphide isomerase (PDI) family is a family of protein folding catalysts found in the ER. PDI itself is the most characterised member of the family but despite this no x-ray structure of full length human PDI (hPDI) has currently been elucidated. Consequently, our understanding of the structural architecture of hPDI comes from limited proteolysis studies, structures of the isolated domains and the high resolution x-ray structure of yeast PDI (yPDI) which has the same domain architecture abb'xa'c.

hPDIp is a pancreas-specific homolog of hPDI found only in pancreatic acinar cells. It is poorly characterised but we expect it to be similar to hPDI in terms of structure as the domain architecture is conserved and the overall sequence identity is ~45%. Given the restricted expression profile of hPDIp we expect that (unlike hPDI), it may have a location-specific role and/or substrate which would thus lead to differences in activity. Furthermore hPDIp has been shown to have quite limited substrate specificity (Klappa et al. 2001, Biochemical Journal 354 553–559) and in addition has a different a' active site motif (CTHC rather than CGHC). Based on these points, we have suggested that the physiological role of hPDIp may be to assist correct folding of pancreatic zymogens.

Our work aims to compare the structure and activities of hPDI and hPDIp. We have already shown that hPDIp is more thermally stable than hPDI although no obvious structural differences have currently been observed. Unexpectedly however, our work has also revealed that unlike hPDI, hPDIp does not undergo major redox-mediated conformational changes. Furthermore, we have shown that hPDIp has ~50% of the oxidoreductase activity, and while this could be improved by mutating the a' active site of hPDIp to CGHC it is possible that other factors account for the remaining loss of activity. This is the current focus of our research where we aim to look at both the chaperone and isomerase activities of hPDIp.

P20r-193
Kinetic and thermodynamic control in the thermal unfolding of bacterial triosephosphate isomerases

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Triosephosphate isomerase (TIM) is a ubiquitous glycolytic enzyme that catalyzes the isomerization of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. All the wild type TIMs so far studied are homooligomers, each monomer folds in to a $(\beta\text{-}\alpha)_8$ barrel. The structure of the TIM barrel is conserved among species, whereas the folding mechanism seems to be sequence dependent. The unfolding of several TIMs, mainly of eukaryotic organisms, has been extensively studied. In most cases the temperaure-induced unfolding, is an irreversible process.

Here, we study TIMs belonging to diverse bacterial phyla: *Deinococcus radiodurans* (*Dr*TIM), Nostoc punctiforme (*Np*TIM), *Gemmata obscuriglobus* (*Go*TIM), *Clostridium perfringens* (CpTIM) and *Streptomyces coelicolor* (*Sco*TIM). All these TIMs were expressed in *E. coli* and purified to homogeneity. Enzymatic activity showed Michaelian kinetics, catalytic efficiency was similar to that reported for other TIMs. Size exclusion chromatography indicated that all the TIMs are dimeric. The thermal unfolding was then studied by Circular Dichroism and Differential Scanning Calorimetry. Unfolding of *Np*TIM and *Go*TIM was irreversible and Tm increased $\asymp$ 5 K when the scan rate was increased from 30 to 180 K/hour, indicating kinetic control; a behavior commonly observed in previously studied TIMs. Interestingly, for *Cp*TIM and *Sco*TIM thermal unfolding was reversible with a hysteresis < 1 K between refolding and unfolding traces, and ΔTm was < 1.5 K; all these evidences are indicative of thermodynamic control. The results presented in this work indicate that for a given topology, the aminoacid sequence determines if the protein is under thermodynamic or kinetic control.

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P20-194
Search for cellular partners of NudC and YgdP Nudix proteins, the potential virulence factors from *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen that frequently causes infections in immunocompromised patients. The observed difficulty in treating infection caused by this microorganism is due to its high intrinsic and acquired resistance against a wide range of antibacterial agents. Thus, there is a need for a search of novel factors involved in *P. aeruginosa* pathogenesis and consequently for novel approaches for controlling these infections. Recently, Nudix proteins were indicated as novel virulence factors of the pathogenic *E. coli* K1, *Legionella pneumophila* and *Pasteurella multocida* strains. Nudix proteins, widely distributed among all classes of organisms, belong to a class of pyrophosphatases. These enzymes catalyze the hydrolysis of a variety of nucleoside diphosphate derivatives including (d)NTPs, NTPs (canonical and modified), nucleoside sugars (e.g., ADP-ribose), various coenzymes, alarmone type molecules ApnA, and m7GTP mRNA cap.

This work focuses on the NudC and YgdP Nudix proteins from *P. aeruginosa*. Based on our previous work and *in silico* analysis these proteins were chosen for studies due to their potential of acting as important cellular regulators that may be involved in pathogenesis of *P. aeruginosa*. During this studies we have established that the NudC Nudix hydrolase of *P. aeruginosa* in the presence of Mg2+ ions and at pH 8.0 preferentially hydrolyses NADH. Using glutaraldehyde as the crosslinking agent we have established that the NudC protein exists in a monomeric form. Due to some technical problems with YgdP purification the biochemical properties of this Nudix hydrolase are still being investigated.

In order to elucidate the biological functions of NudC and YgdP a search for cellular partners of these Nudix hydrolases was performed. Pull-down assay and bacterial two hybrid analysis (BACTH) with NudC and YgdP as the baits and *P. aeruginosa* cellular extracts or *P. aeruginosa* genomic library, respectively, as the source of prays were used. We have identified several proteins of the transcription and translation machinery as the potential interacting partners of these Nudix proteins. Currently, using one-on-one pull-down assays and bacterial two-hybrid analyses, the individual interactions between potential interactors and NudC and YgdP are investigated.

P20-195
Promoter sequence determination of *nudC* gene encoding Nudix-type, novel regulatory growth factor from plant pathogen *Pseudomonas syringae* pv. tomato str DC 3000

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Nudix hydrolases are widely distributed pyrophosphatases with a conserved amino acids motif GX5EX5[UA]XREX2EEXGU. They hydrolyze a variety of nucleoside diphosphate derivatives. The proposed role of Nudix enzymes is to maintain cellular homeostasis. Recently several reports have described some of these proteins from pathogenic bacteria *E. coli* K1, *Legionella pneumophila* i *Pasteurella multocida* as virulence factors playing an important role in pathogen invasion processes.

We have characterized the first Nudix hydrolase from plant pathogen *Pseudomonas syringae* pv. *tomato* str DC 3000. Previously we have established that this 278 aa NudC protein in a presence of Mg^{2+} ions preferably hydrolyzes NADH. We have found that inactivation of the gene encoding the NudC protein severely impairs morphology, growth and swimming, swarming and twitching motility of Pseudomonas cells. Our preliminary results indicate that expression of *nudC* gene is tightly regulated thus there was a need for a search of endogenous promoter of *nudC* gene to study the phenotypic effects of the *nudC* gene expression *in trans*. No information about the promoter sequence of *nudC* gene exists in the Pseudomonas Genome Database V2. In this work an attempt to characterize position of the *nudC* promoter was undertaken. For the genomic region of 3018397–3020318 nucleotides two putative promoter sequences were indicated by the BPROM Softberry Database. These sequences fused to the *nudC* gene were cloned separately into pBBR-MCS3 vector, introduced to the *P. syringae nudC* mutant and the wild type cells and the following phenotypic effects were analyzed.

P20-196
Crystal structure of an outer surface protein BBA64 from *Borrelia burgdorferi* in comparison to BbCRASP-1

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Borrelia burgdorferi is the causative agent of Lyme disease, the most frequent vector-borne disease in Eurasia and North America. Spirochete *B. burgdorferi* is transmitted from infected *Ixodes* ticks to a mammalian host organism during the blood meal. There are several outer membrane lipoproteins whose functions are associated with the pathogenesis of *B. burgdorferi* and which are necessary for the proliferation of the bacteria in an organism. We have solved an x-ray structure of *B. burgdorferi* outer surface lipoprotein BBA64 which expression is significantly up-regulated during the process of tick feeding and has a vital role for the transmission of *B. burgdorferi* from ticks to mammals and could be used as a novel drug target against Lyme disease. A gene coding for *B. burgdorferi* outer surface protein BBA64 was amplified from *B. burgdorferi* strain B31 and the recombinant native and Se-Met labelled proteins with a 6xHis tag and truncated N-terminal signal sequences (1-32) were expressed in *E. coli* and purified by Ni-NTA agarose affinity chromatography and ion-exchange chromatography and crystallized using the sitting drop vapour-diffusion technique. We demonstrate that BBA64 in comparison to a homologous BbCRASP-1 is a monomer in a solution and that the binding site to the yet unidentified ligand for BBA64 is probably very different to that of BbCRASP-1 as the C-terminal part of BBA64 has a different conformation that does not promote the formation of a homodimer which in turn is essential for a proper function of BbCRASP-1.

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P20-197
The C-terminal part of Microcin B is crucial for DNA-gyrase inhibition and antibiotic entry into sensitive cells

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Microcin B (McB), a natural peptide antibiotic produced by *E. coli*, that targets DNA gyrase. McB is synthesized by ribosomes in a form of propeptide – pre-microcin B, which subsequently undergoes post-translational modification. Maturation of microcin B is accomplished by the microcin B synthetase complex McbBCD, which binds the leader peptide of pre-microcin B and converts GS, GC, GSC and GCS sequences into single heterocycles and bis-heterocycles. We have carried out deletion analysis of microcin B, for this purpose 11 derivatives of antibiotic, truncated from the C-terminus, were constructed. Removal of last I^{69} residue decreased bactericidal activity of mutant microcin B derivative (McB1-68) about 50 times. Mutant microcins B with more extended truncations displayed even lower antimicrobial activity, if any. However, analysis of the ability of mutant microcins to inhibit DNA gyrase *in vitro* showed that microcin McB1-68 was only six times less active than it's wild-type counterpart. Mutant microcins with longer deletions (McB1-67, McB1-63HI, McB1-63, McB1-60) were about 90 less active that

the wild-type microcin B in the DNA gyrase inhibition assay. We hypothesized that besides the role in the interaction with DNA gyrase, the last residue I^{69} is also important for microcin B entry into sensitive cells. We support our hypothesis with the results of inhibition of replication in permeabilized *E. coli* cells test. From our data we conclude, that C-terminal residues of McB (from 61 to 69) are important for microcin B inhibition of DNA gyrase and $I^{69}$is also involved in the antibiotic uptake into sensitive cells.

P20-198
Effects of site-directed mutagenesis on the enzymatic activity of non-receptor protein tyrosine phosphatase PTPD1

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Protein tyrosine phosphatases (PTPs) are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle and oncogenic transformation. Protein tyrosine phosphatase D1 (PTPD1) is a cytosolic non-receptor tyrosine phosphatase. PTPD1 can be found in mitochondria [1], along actin filaments, at adhesion plaques [2] and with the endocytic vesicles [3]. PTPD1 primary sequence contains a number of atypical residues within the conserved PTP motifs. It has been reported [4] that PTPD1 has a very low enzymatic activity against a synthetic substrate DiFMUP.

We report here the prokaryotic expression, purification and kinetic evaluation of the wild type PTPD1 as well as of five mutants in which non-conserved residues were back mutated to conserved ones. Purification of the proteins was performed in two steps: affinity chromatography and gel filtration. All proteins were electrophoretically homogenous in SDS-PAGE. Enzymatic characterization of the phosphatase activity of the wild type and mutant proteins was done using DiFMUP as a synthetic PTP substrate. Kinetic analysis was performed both for wild type and mutant PTPD1 forms. The turnover number and the specificity constant were four to seven orders of magnitude larger for all the mutants as compared to the wild type protein.

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P20-199
Spliceosome-associated CTNNBL1 is inessential for cell proliferation but required for rapid exit from quiescence

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CTNNBL1 is an armadillo domain protein that is associated with the spliceosomal CDC5L/PRP19 ('nineteen') complex that is implicated in spliceosome activation. Structurally, CTNNBL1 resembles importin-α and indeed interacts with the nineteen complex by binding, through its armadillo domains, to the NLS of CDC5L. The function of CTNNBL1 is, however, unknown. Here we show that although loss of CTNNBL1 has little effect on the viability or proliferation of cell–lines, CTNNBL1-deficiency is embryonically lethal in mice. Moreover, lineage-specific ablation of CTNNBL1 causes sluggish exit from quiescence in primary cells. This role of CTNNBL1 (dispensable for proliferation but essential for rapid exit from quiescence) also applies in fission yeast and suggests a function in facilitating the rapid activation of RNA splicing that accompanies the cell enlargement and blasting that occurs on exit from quiescence.

P20-200
Purification and characterization of a fibrinolytic serine protease from *Cirriformia tentaculata*

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A novel fibrinolytic enzyme (named CTSP-3) was purified from a marine annelid worm *Cirriformia tentaculata* by three purification steps, including ammonium sulfate precipitation, anion exchange, and size exclusion chromatography in order. The estimated molecular weight of purified enzyme was found to be approximately 28 kDa, as determined by SDS-PAGE. The optimal pH and the temperature for the enzyme activity were found to be 8.5 and 50°C, respectively. The enzyme activity of CTSP-3 was inhibited by PMSF and DFP, suggesting that it is a typical serine protease. The enzyme exhibited an amidolytic activity towards chromogenic substrates, including S-2586, S-2444 and S-2238, demonstrating that it can cleave a peptide bond at the carboxyl side of tyrosine or arginine residues. The enzyme could digest various protein substrates, including azocasein, BSA, prothrombin, plasminogen and fibrinogen. The enzyme could completely cleave the Aα-, Bβ-, and γ-chains of fibrinogen within 10 min and also actively hydrolyze fibrin polymer and cross-linked fibrin. These results suggest that CTSP-3 is a fibrinolytic serine protease that can be used as a thrombolytic agent [This work was supported by a NRF grant of the MEST (J. S. Lee, 20110027675)].

P20-201
Poly-C-binding protein recognition of C-rich oligonucleotides

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Poly-C-binding proteins (PCBPs) are triple KH (hnRNP K homology) domain proteins with specificity for single stranded C-rich RNA and DNA. They play diverse roles in the regulation of protein expression at both transcriptional and translational levels. Here we analyse the contributions of individual PCBP1 KH domains to binding C-rich oligonucleotides using biophysical and structural methods. Using surface plasmon resonance (SPR) we demonstrate that KH1 makes the most stable interactions with both RNA and DNA, KH3 binds with intermediate affinity and KH2 only interacts detectibly with DNA. The crystal structure of KH1 bound to a 5′-CCCTCCCT-3′ DNA sequence shows a 2:1 protein:DNA stoichiometry and demonstrates a molecular arrangement of KH domains bound to immediately adjacent oligonucleotide target sites. SPR experiments, with a series of poly-C-sequences, reveals that cytosine is preferred at all four positions in the oligonucleotide binding cleft and that a C-tetrad binds KH1 with 10 times higher affinity than a C-triplet. The basis for this high affinity interaction is finally detailed with the structure determination of a KH1.W.C54S mutant bound to 5′-ACCCCA-3′ DNA sequence. Together these data establish the

lead role of KH1 in oligonucleotide binding by PCBP1 and reveal the molecular basis of its specificity to a C-rich tetrad.

P20-202
The role of the transmembrane α-helix of human Phospholipid Scramblase 1 in protein structure and function

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Human Phospholipid Scramblase 1 (hPLSCR1) is an endofacial plasma membrane protein that is multipalmitoylated and is widely expressed in most human tissues. hPLSCR1 is involved in the rapid calcium dependent translocation of plasma membrane phospholipids, although neither the detailed calcium-induced conformational change nor the mechanism of phospholipid scrambling are known yet. In addition to this role in phospholipid scrambling, hPLSCR1 may also function to regulate processes including signaling, cell differentiation, apoptosis, injury, cell proliferation and transcription. We have constructed a truncated mutant lacking the transmembrane alpha helix at C-terminal end (C290ΔhPLSCR1) in order to make the protein more stable and soluble. Preliminary structural studies (Fluorescence and Infrared spectroscopies) in the presence of calcium suggest that the truncated binds the ion and undergoes conformational changes similarly to the wild type. Functional studies using the Langmuir balance shows that the ion increases the mutant adsorption to lipid monolayers, and this increase is enhanced by the presence of PS. Nevertheless the protein reconstituted in liposomes does not has any scramblase activity. We conclude that the mutant lacking the transmembrane domain retains two important properties of the native protein, the conformational change due to the calcium binding and the capacity of membrane interaction.

P20-203
Structural dynamics of hemagglutinin complex in botulinum serotype D toxin complex

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Clostridium botulinum produces botulinum neurotoxin (BoNT), a causative agent of the food-borne botulism. In nature, the BoNT forms toxin complex (TC) in conjunction with nontoxic nonhemagglutinin (NTNHA) and/or three types of hemagglutinins (HAs; HA-70, HA-33 and HA-17). L-TC (BoNT/NTNHA/HAs) binds to intestinal epithelial cell and subsequently transports across the cell layer via sugar-chain dependent process. We previously demonstrated that the HA-33/HA-17 complex isolated from the L-TC also bound to the cell and transported the cell layer, indicating that the HA-33/HA-17 complex plays a principal role on the binding and transport of the TC to the intestinal epithelial cell layer. In this study, we performed small-angle X-ray scattering (SAXS) analysis of the HA-33/HA-17 complex isolated from L-TC produced by serotype D strain 4947 (D-4947). Crystal structure of the HA-33/HA-17 complex displayed fan-like structure in which one end of each of two ellipsoidal HA-33 molecules is attached to a single HA-17 molecule with spheral shape. The SAXS image of the HA-33/HA-17 complex exhibited broadly similar appearance of the crystal image of the complex. Superposition of the SAXS and the crystal images of the HA-33/HA-17 complex, however, implied that each HA-33 molecule could move to all directions like a 'joystick' with a pivot of HA-17 molecule. Furthermore, we demonstrated that the SAXS images of the HA-33/HA-17 molecule in the presence of the sugars, *N*-acetylneuraminic acid, glucose and galactose, were significantly altered compared to that in the absence of the sugars. Structural dynamics of the HA-33/HA-17 complex may contribute the cell binding and subsequent transport across the cell layer.

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P20-204
Labeling of hemes for structural studies of cytochromes

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Multi-heme cytochromes are essential redox proteins in sediment organisms that have the ability to perform extracellular electron transfer, such as *Shewanella oneidensis*. Such metabolic ability makes these microorganisms interesting targets for several biotechnological applications, such as bioremediation of metal contaminated environments or production of energy by microbial fuel cells. To understand the electron pathway that connects the cytoplasm to the outer membrane it is important to study the cytochromes involved in this process. We propose the use of hemes labeled in specific carbons as a novel method for the structural and functional characterization of c-type cytochromes, containing multiple hemes. In this work, a well studied four heme cytochrome from *Shewanella oneidensis*, is used to demonstrate the method to label hemes and determine the orientation of the different hemes within the cytochrome. Supplementing an *E. coli* mutant strain, that has no ability to synthesize the heme precursor, δ-aminolevulinic acid (dALA), with dALA specifically labeled will lead to the biosynthesis of hemes labeled in specific carbons. 2-^{13}C-dALA and 5-^{13}C-dALA were used in the biosynthesis of hemes labeled in the methyl and meso carbons, respectively. Hemes labeled in the methyls are useful for the determination of the relative reduction potentials of the hemes. During the titration of multiheme cytochromes, the oxidized fraction of each heme is related to the change in chemical shift of its methyl signals allowing the determination of reduction potentials. Hemes labeled in the mesos carbons allow the determination of residual dipolar couplings (RDC) between the meso protons and the corresponding ring carbon. When the cytochrome is in the oxidized state the unpaired electrons promote the alignment of the molecule in the presence of a strong magnetic field. With four meso per heme the determination of the RDCs of these signals provides information on the relative spatial orientation of the hemes.

P20-205
Magnetic fluids attenuate the cytotoxic effect of amyloid fibrils

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Protein amyloid formation is central to many important human pathologies including Alzheimer's disease, diabetes type II or systemic amyloidosis. A growing body of evidence suggests that the presence of amyloid aggregates is toxic at the cellular level. From this point we have studied effects of magnetic fluid (MF) containing stabilized Fe3O4 magnetic nanoparticles on cells affected by amyloid fibrils. Our results revealed that fibrils are toxic to the cells (V79 and LLC-PK1) and inhibit cell proliferation in a dose- and time-dependent manner. Interestingly, addition of MF to the cell culture media significantly improved viability of cells. On the other hand, Fe3O4 nanoparticles alone did not cause any significant changes in cell viability at studied concentrations. These findings strongly suggest that MF is able to alleviate toxic effect of amyloid fibrils on the cells. We hypothesize that MF is able to decrease the amount of fibrils what translates into reduction of amyloid cytotoxicity. The reported results will contribute to the understanding of the cytotoxic effect of the amyloid fibrils.

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P20-206
Low molecular weight compounds inhibit protein amyloid self-assembly

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Amyloid-related diseases are associated with nonnative folding of proteins and are characterized by the presence of protein amyloid aggregates in the body. Understanding the nature, mechanism and inhibition of amyloid fibril formation facilitates rational drug design for amyloid-related diseases. We have tested a group of various low molecular weight compounds (acridine, phytoalexin and polyphenol derivatives) for their ability to affect insulin/lysozyme amyloid aggregation *in vitro* through the use of spectroscopic and microscopic techniques. The anti-amyloid compound activities were evaluated by the comparison of their median depolymerization concentrations DC50 and median inhibition concentrations IC50. The synergic or antagonistic effects of studied mixtures were also investigated. In addition, possible binding modes of these compounds were analyzed by the use of molecular modeling tools. The structure-activity relationship analysis suggested that structural and conformational features of compounds under study are of key importance for affecting their anti-amyloid activities.

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P20-207
Frankestein RTS-ocellatusin. Evidence for the independent origin of RGD and KTS/RTS disintegrins

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Snake venoms contain a complex variety of pharmacologically active compounds. Disintegrins are a family of small (41–83 amino acids), cysteine-rich polypeptides broadly distributed in the venoms of vipers and rattlesnakes. The disintegrin family comprises potent and specific antagonists of β_1 and β_3 integrin receptors. Their inhibitory activity depends on the appropriate pairing of cysteines, which determines the conformation of an inhibitory loop that harbors an active tripeptide located on a mobile loop. The sequence of the active tripeptide primarily determines the specificity towards integrin receptors. Hence, disintegrins bearing the XXD motif at the tip of an 11-residue mobile loop distinctly block β_1 and β_3 integrins. KTS- and RTS-distintegrins represent selective $\alpha_1\beta_1$ inhibitors. The $\alpha_1\beta_1$ integrin is involved in VEGF-triggered tumor angiogenesis; its selective blockade is thus a desirable goal for killing certain kinds of cancer cells by starving them to death. NMR studies of $\alpha_1\beta_1$-blocking KTS and RTS disintegrins have revealed that their integrin binding loops and C-terminal tails form conformational functional epitopes that display concerted motions. The shape and size of the 9-residue integrin-binding loop, along with its composition, flexibility, and the lateral orientation of the KTS/RTS tripeptide, may underlay the structural basis of RTS/KTS disintegrins' selectivity and specificity for integrin $\alpha_1\beta_1$. We have design, cloned, and recombinantly expressed in soluble form a *Frankenstein* disintegrin in which the RGD motif of ocellatusin is substituted by the RTS tripeptide of jerdostatin. This construct, and an array of subsequent mutants, represent useful tools to investigate the structural basis of the integrin inhibitory potency and anti-angiogenesis activity of $\alpha_1\beta_1$-blocking disintegrins. Moreover, our results provide evidences, that the RTS/KTS short disintegrins have potentially been recruited into the venom gland of Eurasian vipers independently from the canonical neofunctionalization pathway of the RGD disintegrins.

P20-208
Amazing tales about the production of a glycosidase from *Lactobacillus plantarum*

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High efficiency sugar-processing enzymes have been deeply exploited. Much attention has been focused on the use of glycosidases for food industry, particularly, b-D-galactosidases derived from food grade organisms. With this aim, we have produced a novel glycosidase from the lactic acid bacterium Lactobacillus plantarum CECT 748T. We cloned the bgl gene into the pURI3 vector. The recombinant protein His6-Bgl was produced soluble. Although the sequence of the Bgl might indicate the enzyme was a putative b-glucosidase, purified recombinant enzyme showed only b-galactosidase activity against oNPG. Size-exclusion chromatography revealed Bgl behaves in solution as a mixture of

monomeric and a high-molecular weight assembly. The protein was crystallized and structure was solved at 2.4 Å resolution by molecular replacement.

Subsequent attempts for Bgl production failed due to protein precipitation. We investigated new ways for Bgl production and purification. Successful production of Bgl was obtained by designing a mutant (Bgl-2): C211S and C292S. Both Cys residues are fully exposed to the solvent and were identified as covalently modified in the crystal structure what suggests they may be involved in aggregation. Bgl-2 was purified and crystallized rendering good crystals that diffract at 2.5 Å resolution. In order to obtain the soluble wt protein, we have also cloned the bgl gene into the pKLSLt vector which permits producing target proteins N-terminally tagged to the lectin module of the protein LSLa (LSL150). The fusion protein (LSLt-Bgl) was purified by affinity chromatography and the tag subsequently removed by digestion with TEV endoprotease. Untagged Bgl (uBgl) did not precipitate and crystallized.

The characterization of Bgl-2, LSLt-Bgl and uBgl shows they are active against oNPG. Analytical size-exclusion chromatography revealed they behave in solution as an associative system between monomeric and oligomeric species. In addition, thermal denaturation studies using far-UV CD spectroscopy showed a phosphate-stabilizing effect. Solving the structure of Bgl-2 or uBgl in complex with different substrate analogs will help us to elucidate the specifity of the enzyme.

P20-209
Improving the stability of the visual G-protein-coupled receptor rhodopsin in DHA liposomes

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Rhodopsin is the visual photoreceptor responsible for dim light vision. This receptor is located in the rod cells of the retina. It has seven transmembrane helices and is a prototypical member of the G-protein coupled receptors (GPCRs) superfamily.

The structure and function of GPCRs are clearly affected by the lipid composition of the cell membrane. The study of purified recombinant GPCRs is usually done in detergent solution. There is a need to study the receptors in a physiological-relevant milieu because the lipid environment can modulate their function Thus, in this work, rhodopsin has been reconstituted in 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine (DDHA-PC) liposomes - as the retina is rich in DHA- and its thermal stability has been compared with the stability when rhodopsin is solubilised with the detergent *n*-Dodecyl-β-*D*-maltoside (DM).

Rhodopsin thermal bleaching rates, in the dark, were obtained by monitoring the decrease of absorbance at 500 nm as a function of time at 55°C, as an indicator of the percentage of the inactive dark rhodopsin. Rhodopsin in DDHA-PC liposomes showed higher stability than that solubilized in DM. Furthermore, fluorescence spectroscopy also indicated that the active conformation of rhodopsin in DDHA-PC liposomes is preserved and this will allow retinal to re-enter the binding pocket even long time after bleaching. These results demonstrate that a lipid environment -as specific DHA liposomes- can be used to stabilize rhodopsin and its mutants. This strategy will be used in further studies of mutations in rhodopsin associated with congenital retinopathies. Work supported by grant SAF2011-30216-C02-01 (*Ministerio de Ciencia e Innovación*, Spain).

P20-210
Interaction of Thioflavin T with non-polypeptide polyanions – induced circular dichroism study

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Thioflavin T (ThT) – a fluorescence dye – generally used for staining amyloid tissues or detection of amyloid fibrils in solutions. The entrapment of ThT molecules in amyloid fibrils leads to the restraint of free rotation of benzothiazole and benzaminic rings around C-C bond as well as planarization of the conformation. This restriction is essential for occurrence of fluorescence emission in the 475–600 nm region after excitation at 440 nm and also leads to the formation of optically active conformers. The induced optical activity of ThT was also found after binding to α-helical polygutamic acid lacking the β-sheet structure or aromatic side-chains [1]. We have studied the complexes of ThT with non-peptidic polyanions – heparin (HPR), polyadenylate and polyacrylate. Interaction of ThT with polyanions results in entrapment of ThT molecules in uniformly twisted-chiral molecule and possessing the induced circular dichroism, but no fluorescence signal occurs at the same conditions. Stepwise addition of HPR at pH 6.5 leads to occurrence of positive CD band at 408 nm and negative band at 382 nm. In absorption spectra shift from 418 to 385 nm is observed. At higher HPR concentration the sequential vanishing of signals intensities occurs, which can be attributed to the increase spacing between HPR-bound chromophore molecules. Similar results were obtained for polyadenylate. We suggest that ThT may be more unspecific in interactions with not only proteins but also with polymers without regular polypeptide structure.

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P20-211
Rapid oligomer formation of human muscle acylphosphatase induced by heparan sulfate

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Many human diseases are caused by the conversion of proteins from their native state into amyloid fibrils that deposit in the extracellular space. Heparan sulfate, a component of the extracellular matrix, is universally associated with amyloid deposits and promotes fibril formation. The formation of cytotoxic prefibrillar oligomers is challenging to study because of its rapidity, transient appearance and the heterogeneity of species generated. The process is even more complex with agents such as heparan sulfate. We established a new strategy using a stopped-flow device coupled to turbidometry detection that is able to trace the rapid con-

version of human muscle acylphosphatase into oligomers with varying heparan sulfate and protein concentrations in a remarkably reproducible manner. We also analyzed mutants of the 15 basic amino acids of acylphosphatase, identifying the residues primarily involved in heparan sulfate–induced oligomerization of this protein and resolving the process with unprecedented molecular detail. Finally, we showed the general applicability of our method to other protein systems.

P20-212
Ultra fast MAS solid-state NMR studies of protein–protein interactions in the bacterial replisome

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Replication of genomic DNA in bacteria involves multiple stable and transient interactions among protein subunits constituting the replisome. Here we show how ultra-fast magic angle spinning (MAS) solid-state NMR (ssNMR) and ^{1}H-detection on either deutereted or fully protonated samples, can give new information about protein-protein interactions in the *E. coli* replisome. We focused on two fundamental interactions: the first one between the C-terminal domain of the single-stranded (ss) DNA binding protein and its own DNA-binding site, and the second one between the DNA polymerase III subunits α and τ. SSB (a tetramer of 79 kDa) has a structurally well-defined ssDNA binding domain (OB-domain) and an intrinsically disordered C-terminal (Ct) domain. Its extreme Ct acidic motif is known to mediate the binding of SSB to different DNA processing enzymes and scaffold proteins. ssNMR provides, for the first time, residue-specific evidence for interaction, in multiple heterogeneous conformations, of SSB-Ct with its own ssDNA-binding site. In particular we compared spectra from the native protein and from a deletion mutant lacking the extreme Ct. This interaction acts as a switch that directs recruitment of SSB-binding proteins specifically to SSB only when it is bound to ssDNA. Moreover we investigated the interaction between the C-terminal domains of α (α_{CTS} 22.5 kDa) and τ (τ_{C16} 16 kDa). The structure of the complex is so far unknown. We used cell free protein synthesis to produce α_{CTS} in the presence of τ_{C16}. We analyzed two different samples in which each the interacting partners is separately ^{2}H,^{13}C,^{15}N labelled. The complete resonance assignment of the proteins in the complex opens the way to mapping the protein-protein contacts and to the determination of the overall structure.

P20-213
Investigation of a critical radical SAM enzyme involved in co-factor biosynthesis in Actinobacteria

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F420 is a major coenzyme in Archea and Actinobacteria, including *Mycobacterium tuberculosis* [1, 2]. This cofactor is involved in many metabolic pathways; notably, its reduced form protects Mycobacteria from nitrosative stress in macrophages during infection [3]. Hence, the F420 biosynthesis pathway represents an attractive target for drug development against Mycobacteria.

We aimed to characterize the penultimate reaction of F420 biosynthesis catalyzed by the enzyme FO-synthase, which is part of the superfamily of radical S-adenosylmethionine (SAM) enzymes. These enzymes catalyze the reductive cleavage of SAM bound to their [4Fe-4S] cluster held by a characteristic SAM radical motif: CxxxCxxC. In Mycobacteria, FO-synthase contains two radical SAM motifs whereas in methanogenic Archea, FO synthase is composed of two subunits. This implies that two radical species are likely generated during catalysis to perform the complex reaction catalyzed by FO synthase. Using physico-chemical techniques approaches combined with mutagenesis study we investigated in details the reaction mechanism of this new radical SAM enzyme. The knowledge gained on the mechanism of this enzyme not only brings new insight into the radical SAM enzyme superfamily but also on the physiology of Archae and Actinobacteria, including the human pathogen *M. tuberculosis*.

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P20-214
The effects of ligand binding on protein structure and rigidity: studies with HIV-1 protease and cyclophilin A

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For certain proteins there are a large number of crystal structures available, making them appropriate as data sources for studying the effects of ligand binding on their structure and function. The rigidity analysis software FIRST divides a protein into rigid and flexible regions based on a bond network inferred from a crystal structure. FIRST is computationally inexpensive, allowing the investigation of multiple structures in tandem. We have evaluated 206 high-resolution (≤2 Å) X-ray crystal structures of HIV-1 protease and used FIRST to compare the effects of different inhibitors on the rigidity of the enzyme. We find that inhibitor binding has little effect on the overall rigidity of the protein homodimer, including the rigidity of the active site and that the principal effect of inhibitor binding on rigidity is to constrain the flexibility of the beta-hairpin flaps, which move to allow access to the active site of the enzyme. We show that commercially available antiviral drugs which target HIV-1 protease can be divided into two classes, those which significantly affect flap rigidity and those which do not. The non-peptidic inhibitor tipranavir is distinctive in its consistently strong effect on flap rigidity. We have also evaluated the rigidity of 54 structures of the peptidyl prolyl cis-trans isomerase cyclophilin A and are currently comparing the results of these rigidity analyses with experimental findings. The techniques used include HD exchange, fluorimetry, and circular dichroism. Initial results are presented in addition to details of currently ongoing experiments.

P20-215
Quantifying the effect of aminoacid composition on protein folding kinetics and stability

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It is basic knowledge that the aminoacid sequence is the ultimate factor in specifying and governing the formation of the native structure. However, protein folding rates seem to largely depend on much coarser properties such as size, and topology. A number of successful methods that have been developed over the last years to predict folding rates based on these inputs still result in predictions with significant scatter. Such scatter only reflects the fine-tuning due to the specific sequence. Other properties that depend on the sequence are effects of mutations and distinction between proteins that fold and those that are intrinsically disordered (IDPs). We recently implemented size and structural class (10-bits) description to a physics-based model of protein folding (PREFUR) that rendered remarkably accurate rate predictions for a database of 52 proteins.

Here, we extend it to investigate the role of aminoacid composition in determining folding and unfolding rates, stability, mutational effects, and whether the protein is an IDP. To do so we have implemented our basic free energy surface model with a simplistic force-field that only depends on the aminoacid composition (not on the sequence or the structure) and improved the size-scaling features of the model by accounting for formation of sub-domain architecture on domains larger than 110 residues. This new model reaches accuracies closer to ± 0.34 and ± 0.7 for folding and unfolding rates and around 5 kJ/M for stabilities that are comparable in magnitude to the effects of single-point mutations. It also identifies previously characterized IDPs. Since the method only uses aminoacid composition and size, it can be directly applied for scanning the vast protein sequence databases and analyzing entire proteomes.

P20-216
Novel methyl transfer reactions: expanding the chemistry of radical SAM enzymes

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Methylation is among the most widespread chemical modification encountered in biomolecules from small organic compounds to large macromolecules such as proteins and DNA. Despite its simple structure, it plays a pivotal and critical role in many major biological processes including epigenetics, cancer or bacterial resistance to antibiotics. Enzymes catalysing methyl transfer reactions (i.e. methyltransferases) have been widely studied and the wide majority of them use a simple mechanism which proceed via an S(N)2 displacement and the use of S-Adenosyl-L-Methionine (AdoMet) as methyl donor.

Recently, a new family of enzymes, using a radical-based mechanism, has been identified as catalyzing a broad range of chemically difficult reactions. This large super-family of enzymes, because of their unusual mechanism employing SAM and radical chemistry, was called radical *S*-Adenosyl-L-Methionine or radical SAM enzymes.

We uncovered a new radical SAM enzyme which use an unprecedented chemistry to methylate inactivated sp^2 hybridized carbon atoms. Further enzyme characterization show that it might produce a methyl radical, a unique feature in biochemistry.

Keywords: Radical SAM, Methylation, Antibiotic, iron-sulfur enzyme.

P20-217
Insight into a new type of methyltransferase belonging to the radical AdoMet enzyme superfamily: the antibiotic resistance enzyme Cfr from *Staphylococcus aureus*

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Various enzymes are involved in bacterial resistance mechanisms to antibiotics. The gene *cfr* is a new multi-drug resistance gene discovered in *Staphylococcus aureus* strains. This *cfr* gene confers resistance to several structurally unrelated antibiotics targeting the bacterial ribosome peptidyl transferase center. This gene may encode a new type of methyltransferases which methylates the nucleotide A2503 of *Escherichia coli* 23S rRNA subunit [1]. We have undertaken the *in vitro* characterization of Cfr in order to better understand this new bacterial resistance mechanism. Our data unambiguously demonstrate that Cfr belongs to the radical *S*-adenosyl-L-methionine (SAM) enzyme superfamily. This large family of enzymes (more 3000 members) catalyzes many different reactions such as cofactor synthesis, DNA reparation or RNA modification. The presence of a [4Fe-4S] cluster coordinated by three cysteinyl residues in a conserved CX_3CX_2C motif is a main characteristic of this family. Its function is to bind and reductively cleave SAM to form the highly reactive 5′ deoxyadenosyl radical which eventually proceeds to the reaction product [2].

Furthermore we demonstrated that the transfer of a methyl group to a minimal RNA substrate is catalyzed by Cfr making it unique among the emerging super-family of radical SAM enzymes. The critical role for additional conserved cysteinyl residues was confirmed by mutagenesis experiments. In addition, we also demonstrated that two SAM molecules are necessary for one RNA methylation reaction. The first molecule of SAM serves for reaction initiation and the second SAM is used as an indirect methyl donor. Labeling experiments further demonstrated that Cfr catalyzes an unusual methyl transfer reaction with a new mechanism.

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P20-218
Synergic and opposing activities of thermophilic RecQ-like helicase and Topoisomerase 3 in Holliday junction processing and replication fork stabilization

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RecQ family helicases and Topoisomerase 3 enzymes form evolutionary conserved complexes which play essential functions in DNA replication, recombination and repair, and in vitro show coordinate activities on model recombination and replication intermediates. Malfunctioning of these complexes in humans is associated with genomic instability and cancer-prone syndromes. Although both RecQ-like and Topoisomerase 3 enzymes are present in archaea, only a few of them has been studied and no information about their functional interaction is available. We tested the combined activities of the RecQ-like helicase, Hel112, and the Topoisomerase 3, SsTop3, from the thermophilic archaeon Sulfolobus solfataricus. Hel112 showed coordinate DNA unwinding and annealing activities, a feature shared by eukaryotic RecQ homologs, which resulted in processing of synthetic Holliday junctions and stabilization of model replication forks. SsTop3 catalysed DNA relaxation and annealing. When assayed in combination, SsTop3 inhibited the Hel112 helicase activity on Holliday junctions and stimulated formation and stabilization of such structures. In contrast, Hel112 did not affect the SsTop3 DNA relaxation activity. RecQ-Topoisomerase 3 complexes show structural similarity with the thermophile-specific enzyme reverse gyrase, which catalyzes positive supercoiling of DNA and was suggested to play a role in genome stability at high temperature. Despite such similarity and the high temperature of reaction, the SsTop3-Hel112 complex does not induce positive supercoiling and is thus likely to play different roles. We propose that the interplay between Hel112 and SsTop3 might regulate the equilibrium between recombination and anti-recombination activities at replication forks.

P20-219
Characterization of the unfolding processes of the tetrameric and dimeric forms of *Cratylia mollis* seed lectin (CRAMOLL 1): effects of natural fragmentation on protein stability and biological activity

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pCRAMOLL 1 is a major isolectin found in seeds of *Cratylia mollis* (Leguminosae family, Diocleinae subtribe). The lectin (236 amino acids) shares with concanavalin A the same pH dependent dimer–tetramer equilibrium and the ability to recognize Glc/Man moieties. Intricate post-translational events occurring in Diocleinae seeds result in a mixture of intact and fragmented monomers within the oligomeric assemblies of pCRAMOLL 1. We have demonstrated that the bacterial recombinant form of CRAMOLL 1 (rCRAMOLL 1) retained sugar-binding activity and several biophysical properties of pCRAMOLL 1, but its tetramers, which are composed of intact monomers only, show enhanced stability when probed with acidification and increased urea concentrations. Using fluorescence, circular dichroism, size-exclusion chromatography, and chemical cross-linking experiments, we posited that the absence of fragmentation gives to the tetramers a higher thermodynamic stability, what does not take place in the case of the dimers. While the dimer and tetramer of pCRAMOLL 1 unfolded via a compact monomeric intermediate, the recombinant tetramer (but not its dimer) remarkably showed no evidence of such dissociation. From our studies, we envision that the fragmentation that takes place in plant cells could modulate lectin activity by affecting the oligomerization state and therefore its sugar binding capacities. Now, we are testing if this modulation occurs *in vivo* by comparing the performance of p and rCRAMOLL 1 to agglutinate and kill *Leishmania* promastigotes, stimulate Th1 cell response and heal footpad lesions in a murine model of cutaneous leishmaniasis.

P20-220
The tumor suppressor protein p53 is structurally stabilized by a double-stranded DNA thioaptamer and protected from heat and high pressure denaturation

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The transcription factor p53 is mutated in around 50% of all tumors. These missense mutations usually lead to DNA binding incapability or to the expression of unstructured proteins. The stabilization of p53 structure, with the recovery of its function, is a promising strategy for cancer therapy. Moreover, recent studies have shown that many p53 hot-spot mutations have a tendency to undergo aggregation, trapping both wild type and mutant forms, and even including p53 paralogs, such as p63 and p73. These new data suggest that cancer could also be considered a protein misfolding disease, which unravels a new pathway to the development of new drugs for cancer chemotherapy. Thioaptamers are nucleic acids with phosphorothioate modifications that confer nuclease resistance and enhance binding affinity to proteins. p53 acts as a transcription factor through the recognition of consensus sequences present in the genomic DNA. In this work, a double-stranded 21-bp consensus sequence modified to be a thioaptamer was used as a tool for the rescue of p53 central core domain (p53C) structure. The thioaptamer has increased the stability of p53 against pressure denaturation. It has also protected p53C from aggregation at 37°C. In addition, experiments with *hot-spot* p53 mutants R248Q and R175H have been performed. Our results show that the consensus DNA thioaptamer was able to recover p53 structure after high pressure and temperature perturbations. These modified molecules are promising candidates for cancer chemotherapy through the rescue of p53 from aggregation.

P20-221
Identification of a novel Baeyer-Villiger monooxygenase from *Acinetobacter radioresistens*: close relationship to the *Mycobacterium tuberculosis* prodrug activator EtaA

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This work demonstrates that *Acinetobacter radioresistens* strain S13 during the growth on medium supplemented with long chain alkanes as the sole energy source expresses *alm*A gene coding for a Baeyer-Villiger monooxygenase (BVMO) involved in alkanes subterminal oxidation. Phylogenetic analysis placed the sequence of this novel BVMO in the same clade of the prodrug activator ethionamide monooxygenase (EtaA) and it bears only a distant relation to the other known class I BVMO proteins. *In silico* analysis of the 3D model of the S13 BVMO generated by homology modelling also supports the similarities with EtaA by binding ethionamide to the active site. *In vitro* experiments carried out with the purified enzyme confirm that this novel BVMO is indeed capable of typical Baeyer-Villiger reactions as well as oxidation of the prodrug ethionamide.

P20-222
Structural studies of the protein machinery for DNA processing and translocation in bacterial conjugation

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Whatever the route used, horizontal gene transfer requires sophisticated multi-protein machinery to enable the long and charged DNA polymer to cross the cell envelope barriers. The best-studied system for cell-to-cell DNA translocation is bacterial conjugation, a major mechanism for genetic exchange in bacteria, which provides a route for the rapid acquisition of new genetic information and contributes to the spread of antibiotic resistance. Over the last decades research efforts in the field have resulted in the clarification of many aspects of this system and its machinery assembly. The goal of our work is to explain based on structural biology the mechanism of action of some of the missing pieces of this phenomena. These factors include MobM relaxase encoded on the Gram-positive streptococcal plasmid pMV158, as well as TrwC relaxase/helicase and TrwK protein (VirB4 homologue), the largest type IV secretion system component, both from the Gram-negative plasmid R388. Progress in the expression, purification, crystallization and structural characterization of these proteins will be presented.

P20-223
Structural characterization of the recombinant importin-α from *Neurospora crassa*

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Neurospora crassa has been widely used as a model organism and contributed to the development of biochemistry and molecular biology by allowing the identification of many metabolic pathways and mechanisms responsible for gene regulation. Nuclear proteins are synthesized in the cytoplasm and need to be translocated to the nucleus to exert their functions which the importin-α receptor has a key role for the classical nuclear import pathway. In an attempt to get structural information of the nuclear transport process in *N. crassa*, we present herein the cloning, expression, purification and structural studies with N-terminally truncated importin-α from *N. crassa* (IMPα-Nc). Circular dichroism analysis revealed that the IMPα-Nc obtained is correctly folded and presents a high structural conservation compared to other importins-α. Dynamic light scattering, analytical size-exclusion chromatography experimentsand molecular dynamics simulations indicated that the IMPα-Nc unbound to any ligand may present low stability in solution. The IMPα-Nc theoretical model displayed high similarity of its inner concave surface, which binds the cargo proteins containing the nuclear localization sequences, among IMPα from different species. However, the presence of non-conserved amino acids relatively close to the NLS binding region may influence the binding specificity of IMPα-Nc to cargo proteins.

P20-224
Evidence for *in vivo* expression of shewasins A and D, two pepsin homologs from bacteria

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The presence of genes encoding pepsin-like enzymes in bacteria has been recently demonstrated by bioinformatics analys [1] and by our work on the recombinant expression of *Shewanella amazonensis* pepsin homolog (shewasin A) [2] that provided the first documentation of this type of activity in prokaryotes. These evidences established beyond doubt that pepsin-like enzymes are not confined to eukaryotes.

The goal of this study was to evaluate the expression of native shewasin A and to expand these studies to *Shewanella denitrificans* where a hypothetical pepsin homolog gene was also identified.

Total RNA extracts isolated from *S. amazonensis* and *S. denitrificans* were used for RT-PCR analysis. The results obtained demonstrate that both genes are transcribed *in vivo* in these bacteria. An antibody raised against *S. denitrificans* pepsin homolog (shewasin D) was used to evaluate protein expression. Again, our results showed that native shewasin D is abundant in *S. denitrificans* cell extracts. The protein was partially purified by Pepstatin A-agarose, further demonstrating its affinity for the specific inhibitor of aspartic proteases. The recombinant form of shewasin D was then produced in *E. coli* for further characterization. As observed for shewasin A, recombinant shewasin D properties fitted those of pepsin-like enzymes, being maximally active at acidic pH, inhibited by pepstatin and displaying similar specificity requirements.

These results provide the first evidences of *in vivo* expression of pepsin-like homologs in two species of bacteria and clearly suggest that this type of activity appears to be more widespread in prokaryotes than initially anticipated, raising new questions on the functional relevance of shewasins.

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P20-225
The Wss1 Zn-dependent metalloprotease interacts genetically with the Ulp2 poly-SUMO specific isopeptidase

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The *Wss1* (weak suppressor of *smt3* encoding SUMO) protein is a novel SUMO-dependent isopeptidase from the yeast *Saccharomyces cerevisiae*. Here, we show that Wss1p displayed *in vitro* Zn^{2+} dependent metallopeptidase activity, inhibited by metalloprotease inhibitors or mutations in the HELXH(X_5)H metallopeptidase motif of Wss1p. Deletion of *WSS1* resulted in increased sensitivity to DNA damaging agents. Expression of the Wss1-A115/A119 variant with the mutated metallopeptidase motif failed to restore the wild-type phenotype to the *wss1*Δ cells, indicating that the protease activity was required for the cell response to DNA damage. Expression of the wild-type or Wss1-A115/119 prevented the accumulation of high molecular weight SUMO-conjugates seen in the *ulp2* strain, which is defective in a poly-SUMO chains isopeptidase. This reduction was not seen in the cells expressing Wss1ΔSIM, a C-terminally truncated variant lacking a SUMO-interacting motif (SIM). Phenotypic analysis also showed that Wss1p and Wss1-A115/A119, but not Wss1ΔSIM, suppressed the growth defects of the *ulp2* strain at 37°C. These results indicate that Wss1p is able to substitute for the absence of Ulp2p, and this function required binding to SUMO chains. Surprisingly, the *WSS1* deletion also suppressed the growth defect of the *ulp2* (or *ulp1*) strain at 37°C and this effect correlated with the reduced levels of poly-SUMO conjugates in *wss1*Δ *ulp2*Δ (*wss1*Δ *ulp1*Δ), compared to *ulp2*Δ (*ulp1*Δ). The complex genetic interaction with Ulp2p reveals a dual role for Wss1p in the regulation of free SUMO levels. Biochemical fractionation experiments showed association of Wss1p with membranes. A Wss1-GFP fusion protein showed 1–4 foci in the nucleus of wild-type and *ulp2* cells. In contrast, depending on the growth conditions a diffuse nuclear signal or ER fluorescence was seen in *uba2* cells, which are defective in SUMO activation prior to subsequent conjugation to target proteins. This result indicates that subcellular localization of Wss1p requires the binding to unidentified sumoylated nuclear proteins or its covalent attachment to SUMO.

P20-226
Avian reovirus takes advantage of apoptosis to produce several muNS isoforms

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The Orthoreovirus Genus comprises nonenveloped viruses with a double stranded RNA genome of ten segments with a double protein capsid shell. The two main classes of this Genus are mammalian reovirus (MRV) and avian reovirus (ARV). These viruses replicate within cytoplasmic globular structures termed viral factories, the matrix of which is formed by the nonstructural protein muNS (Benavente and Costas, 2007). Two muNS isoforms are expressed in infected cells by both MRV and ARV. The muNS isoforms of MRV are polypeptides of 80 and 75 kDa, while the ones of ARV are polypeptides of 70 and 55 kDa. (Tourís-Otero F., 2004) (Wiener, 1989). The larger isoforms are encoded by the longest open reading frame of their respective M3 genes. The smaller isoforms could originate by internal translation initiation at an in-frame start codon or by partial proteolytical cleavage.

Several lines of evidence indicated that the smaller MRV muNS isoform originates by secondary initiation at in-frame AUG codon 41 of the MRV m3 mRNA (Kobayashi, 2006) (Arnold, 2008) (Busch, 2011), and this isoform was called muNS-C to as it belongs to the C-terminus of muNS. Recent results from our laboratory have demonstrated that the smaller ARV muNS isoform originates by a specific posttranslational cleavage, generating an N-terminal peptide of ~15 kDa (termed muNSN) and a C-terminal polypeptide of 55 kDa (termed muNSC). Surprisingly, this processing is not detected in transfected cells or in insect cells infected with a recombinant baculovirus that expresses ARV muNS, suggesting that its cleavage is promoted by the reoviral infection.

In this study we present evidence that muNS is cleaved by caspases activated during ARV infection and that cleavage occurs between residues Asp-154 and Ala-155 of ARV muNS

P20-227
The magic spot ppGpp regulates the molecular and functional properties of the elongation factor 1α from the archaeon *Sulfolobus solfataricus*

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Guanosine tetra-phosphate (ppGpp) is a key molecule in the stringent control of few strains of halophilic and methanogenic archaea but not hyperthermophilic ones. Here we show that ppGpp is able to affect the functional and molecular properties of the archaeal elongation factor 1α isolated from *Sulfolobus solfataricus* (SsEF-1α). Indeed, ppGpp inhibited archaeal *in vitro* protein synthesis, even though the concentration required to get inhibition was higher than that required for the eubacterial and eukaryal systems. Regarding the partial reactions catalyzed by SsEF-1α, the effect produced by ppGpp on the affinity for aa-tRNA was lower than that measured in the presence of GTP but higher than that for GDP. The magic spot I was also able to bind SsEF-1α as demonstrated by its ability to displace the GDP bound to the elongation factor. Also in this case the affinity of the protein for ppGpp was intermediate in comparison to that displayed by GDP and GTP. Furthermore, ppGpp inhibited the intrinsic GTPase catalysed by SsEF-1α with a competitive behavior. Finally, the binding of ppGpp to SsEF-1α rendered the elongation factor more resistant to heat treatment as the temperature for half-denaturation was increased by 2°C. The analysis of the molecular model of the complex between SsEF-1α and ppGpp suggests that this stabilisation arises from the charge optimisation on the surface of the protein. The data here reported pointed to the finding they the binding of ppGpp, characterised by struc-

tural requirements similar to those required by GDP, could regulate some cellular processes without interfering with the GDP/GTP balance which is important for many other metabolic ways.

P20-228
Use of peptides to investigate the binding sites of the diacylglycerol acyltransferase1 enzyme

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The diacylglycerolacyltransferase1 (DGAT1) is the key enzyme in the triacylglycerol synthesis in diverse living organisms. Since the knowledge of detailed structural information about this enzyme is hampered by its high hydrophobic character and many transmembrane domains, the aim of this study was to use synthetic peptides corresponding to DGAT1 putative binding sites (Sit1 and Sit2) to investigate the interaction with substrates and different membrane models (monolayers, vesicles and micelles). After synthesis, purification and mass spectrometry characterization, Sit1 showed to bind more effectively into the internal region of the micelles and in lipid monolayers at low packing, while Sit2 bound strongly to negatively charged surfaces, with an unordered to helix transition in circular dichroism spectra and a 20 nm blue shift in fluorescence emission. The binding of Sit1 and Sit2 peptides to negative liposomes gave dissociation constants (K_D) of 170 and 0.44 υM, measured in a BIACORE system, respectively, and a leakage action 24-fold higher to Sit2. The difference in binding observed in the membrane models was in agreement with the interaction observed with oleyl-CoA and dioleoylglycerol, in which Sit1 showed to bind to the most nonpolar substrate and Sit2 binds the negatively charged substrate. The knowledge of the DGAT1 binding sites structure and the determination of the main regions requested for its activity, certainly contribute to the investigation of the enzyme mechanism of action. Supported by FAPESP and CNPq.

P20-229
Antibody – encoded primitive active site mediates the conversion of organophosphorus compounds via covalent catalysis

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Immunoglobulins were shown to serve as a good template to generate de novo catalytic active centers by combinatorial and rational design approaches. We have used a strategy of covalent selection in order to probe for inherently reactive antibody V regions and isolate these V region products through the strong covalent binding interaction from human semi-synthetic phage display library. The selected scFv antibody, containing nucleophilic Tyr37 was able to capture a set of organophosphorus compounds. It was converted into the full-size antibody and expressed in CHO cells. Fab-fragment was crystallized and 3D structures of apo- and phosphorylated by initial hapten antibodies were solved with resolution 1.5 A and 1.36 A respectively. The observed pre-existing primitive active site with acetyl cholinesterase-like deep cavity was shown to hydrolyze pesticide paraxon via covalent catalysis. The nucleophile competition effect allowed estimating elementary constants of three-stage kinetic scheme. The dephosphorylation was shown as a rate-limiting step. The site-directed mutagenesis revealed that the second shell interactions play key role for binding specificity.

P20-230
Identification of allosteric residues important for substrate-stimulated ATPase mechanism in the ATPase domain of Hsp70 molecular chaperones

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Hsp70 molecular chaperones have critical function in cells. Hsp70s consist of a conserved N-terminal ATPase domain (NBD) and a C-terminal substrate-binding domain. Interdomain communication between the domains is essential for Hsp70 chaperone function and studies done on an *Escherichia coli* homolog, DnaK, revealed that a conserved linker between the domains is important for the allosteric signaling. When linker interacts with NBD, studied by the construct containing the entire linker, DnaK (1–392), an enhanced ATPase rate is observed compared to the construct lacking the conserved ^{389}VLLL^{392} linker region, DnaK (1–388). This observation suggests that structural rearrangements caused by linker docking adopt the ATPase domain in a closed conformation, leading to an enhanced, pH-dependent ATPase activity. Here, our aim is to delineate the residues that are responsible for the linker induced conformational rearrangements. In that line, using molecular dynamic simulations we identified two sets of amino acids at the lobe interface of the ATPase domain that might be critical in the stabilization of the domain in the so called 'open' and 'closed' conformations. We made point mutations for these sites on both DnaK (1–392) and DnaK (1–388) constructs, and studied the structural and functional effects of these residues on the ATPase domain using pH varied stability measurements by circular dichroism and activity measurements as a function of pH, respectively. Limited trypsinolysis experiments were performed to study the dynamic variations on the ATPase domain caused by mutations. Our experimental results also point to the significance of these residues in the domain rearrangements when triggered by linker binding.

P20-231
Spectroscopic characterization of a trimeric β-sheet-rich intermediate state that populates during PSD95-PDZ3 misfolding

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The temperature-induced misfolding pathway of the third PDZ domain of the PSD95 neuronal protein, PDZ3, is populated by a trimeric β-sheet-rich intermediate state. This intermediate state organizes in a stepwise and reversible way the formation of different supramacromolecular structures (1). In this work, we have undertaken FTIR, TEM, NMR and cytotoxicity experiments, under neutral pH conditions, to characterize the conformational transition that leads PDZ3 to misfolding. FTIR results have shown that the misfolding pathway is not due to different ensembles of a variety of precursors, but mainly comes from the interconversion of a flexible β-sheet of the domain into worm-like fibrils, which have been confirmed by TEM micrographs. Toxicity assays in a human neuroblastoma cell-line SH-SY5Y show that cytotoxicity increases as the aggregation pathway proceeds, suggesting that the pathway is only partially reversible. Finally, NMR analysis of chemical shifts as a function of temperature has allowed us to establish the β-sheet arrangement around strand β3 as the promoter of the conformational change that drives PDZ3 domain to misfolding.

P20-232
KH domains with impaired nucleic acid binding as a tool for functional analysis

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In eukaryotes, RNA binding proteins that contain multiple KH domains are key to coordinate the different steps of RNA synthesis, metabolism and localisation. Understanding how the different KH modules participate in the recognition of the RNA targets is necessary to dissect the way these proteins operate. We have designed a general use KH mutant with impaired RNA binding capability to explore the role of individual KH domains in the combinatorial functional recognition of RNA targets. A double mutation in the hallmark GxxG loop (GxxG-to-GDDG) impairs nucleic acid binding without compromising the stability of the domain. We analysed the impact of the GDDG mutations in individual KH domains on the functional properties of KSRP as a prototype of multiple KH domain-containing proteins. We show how the GDDG mutant can be used to directly link biophysical information on the sequence specificity of the different KH domains of KSRP and their role in mRNA recognition and decay. This work defines a general molecular biology tool to investigate the function of individual KH domains in nucleic acid binding proteins.

P20-233
Kinetic investigations on the reaction of a 2-oxoglutarate dependent histone demethylase with oxygen

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Histone lysine demethylases comprise an important family of epigenetic regulatory enzymes. They catalyse the demethylation of tri-, di- and monomethylated lysine residues of histones, thus contributing to either silencing or activation of chromatin (1). Most histone lysine demethylases belong to the 2-oxoglutarate and ferrous iron dependent dioxygenase superfamily. This family utilises molecular oxygen to catalyse the hydroxylation of substrates, in a process coupled with 2OG decarboxylation (2). The histone demethylase KDM4E acts on tri- and dimethylated H3K9, has the structural features typical of Fe(II)/2OG oxygenases (3).

We report kinetic studies on the oxygen dependence of KDM4E. Steady state assays show KDM4E has a graded response to O_2 over a physiologically relevant range of O_2 concentrations. Pre-steady state assays imply that KDM4E reacts slowly with O_2 and that there are mechanistic variations dependent on the methylation status of the substrate. The results demonstrate the potential for histone demethylase activity to be regulated by oxygen availability.

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P21 – Regulation of Gene Expression and Genome Dynamics

P21-1
Control of Ubp3 ubiquitin protease activity by the Hog1 SAPK modulates transcription upon osmostress

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Protein ubiquitylation is a key process in the regulation of many cellular processes. The balance between the activity of ubiquitin ligases and that of proteases controls the level of ubiquitylation. In response to extracellular stimuli, stress-activated protein kinases (SAPK) modulate gene expression to maximize cell survival. In yeast, the Hog1 SAPK has a key role in reprogramming the gene expression pattern required for cell survival upon osmostress. Here, we show that the Ubp3 ubiquitin protease is a target for the Hog1 SAPK to modulate gene expression. ubp3 mutant cells are defective in expression of osmoresponsive genes. Hog1 interacts with and phosphorylates Ubp3 at serine 695, which is essential to determine the extent of transcriptional activation in response to osmostress. Furthermore, Ubp3 is recruited to osmoresponsive genes to modulate transcriptional initiation as well as elongation. Therefore, Ubp3 activity responds to external stimuli and is required for transcriptional activation upon osmostress.

P21-2
Non-catalytic functions of DNMT1: modulation of the transcriptional regulation of E-cadherin by SNAIL1

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Mammalian DNA methyltransferase 1 (DNMT1) is essential for maintaining DNA methylation patterns after cell division. Disruption of DNMT1 catalytic activity results in whole genome cytosine demethylation of CpG dinucleotides, promoting severe dysfunctions in somatic cells and during embryonic development. While these observations indicate that DNMT1-dependent DNA methylation is required for proper cell function, the possibility that DNMT1 has a role independent of its catalytic activity is a matter of controversy. Here we provide evidence that DNMT1 can support cell functions that do not require the C-terminal catalytic domain. We report that PCNA and DMAP1 domains in the N-terminal region of DNMT1 are sufficient to modulate E-cadherin expression in the absence of noticeable changes in DNA methylation patterns in the gene promoters involved. Changes in E-cadherin expression are directly associated with regulation of β-catenin-dependent transcription. Present evidence suggests that the DNMT1 acts on E-cadherin expression through its direct interaction with the E-cadherin transcriptional repressor SNAIL1.

P21-3
Regulation of histone modifying enzymes by vitamin D in human colorectal cancer cells

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Vitamin D from the diet or synthesized in the skin upon UV-B irradiation is converted into the active metabolite 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Epidemiological studies have shown that $1,25(OH)_2D_3$ has protective effects against colorectal cancer (CRC). Histone tails are subjected to a variety of post-translational modifications whose misregulation may contribute to cancer. We report that $1,25(OH)_2D_3$ has an unanticipated wide regulatory action on the expression of genes coding for histone demethylases of the Jumonji C (JmjC) domain and lysine-specific demethylase (LSD) families. *JMJD3* gene encodes a histone H3 lysine 27 (H3K27) demethylase proposed to have tumour suppressor activity. $1,25(OH)_2D_3$ induces *JMJD3* RNA expression and activates its gene promoter in human colon cancer cells. In SW480-ADH cells, *JMJD3* knockdown decreases the induction by $1,25(OH)_2D_3$ of *CYP24A1*, *CDH1* and *CST5* genes and of an epithelial adhesive phenotype. Moreover, *JMJD3* knockdown upregulates the epithelial-mesenchymal transition inducers *SNAI1*, *ZEB1* and *ZEB2* and the mesenchymal markers *FN1* and *LEF1*, while it downregulates the epithelial genes *CDH1*, *CLDN1* and *CLDN7*. Also, *JMJD3* knockdown decreases the expression of *miR-200b* and *miR-200c*, two microRNAs targeting *ZEB1* RNA. *JMJD3* knockdown blunts the nuclear export of β-catenin and abolishes the inhibition of β-catenin transcriptional activity caused by $1,25(OH)_2D_3$. In addition, the expression level of *JMJD3* RNA is lower in tumour than in normal tissue in 56% of the CRC patients studied. Together, our results indicate that (1) $1,25(OH)_2D_3$ regulates the expression of several histone modifying enzymes involved in epigenetic regulation and (2) the histone demethylase *JMJD3* is a VDR target that partially mediate the effects of $1,25(OH)_2D_3$.

P21r-4
MicroRNA-22 mediates partially the effects of Vitamin D3 in colon cancer

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Vitamin D deficiency is associated with high risk of colon cancer. The most active vitamin D metabolite, 1α,25-dihydroxyvitamin

D3 ($1,25(OH)_2D_3$) induces differentiation, sensitizes cells to apoptosis, and inhibits the proliferation of cultured human colon carcinoma cells through the regulation of the transcription rate of numerous target genes *via* its nuclear receptor, VDR. MicroRNAs (miRNAs) are short non-coding RNAs that have been shown to play key roles in cancer controlling the expression of oncogenes and tumour suppressor genes. We have identified miR-22 and other miRNA species as $1,25(OH)_2D_3$ targets in human colon cancer cells. MiR-22 is induced by $1,25(OH)_2D_3$ in a time-, dose-, and VDR-dependent manner. We have studied the effect of miR-22 on the phenotype and gene expression of SW480-ADH and HCT116 cells. MiR-22 loss-of-function by transfection of a miR-22 inhibitor suppresses the antiproliferative effect of $1,25(OH)_2D_3$. Also, miR-22 inhibition increases cell migration *per se* and decreases the antimigratory effect of $1,25(OH)_2D_3$. Furthermore, *in silico* analyses have showed a significant overlap between genes repressed by $1,25(OH)_2D_3$ and miR-22 putative target genes. We found that miR-22 mediates the repression by $1,25(OH)_2D_3$ of *OGN*, *NELL2*, *HNRPH1*, *RERE* and *NFAT5* genes in these cells. Finally, we studied the expression of miR-22 in 50 matched normal and tumour samples from human colon cancer patients. In 78% patients, miR-22 expression was found lower in the tumour than in the matched normal tissue and correlated directly with that of *VDR*. In conclusion, our results indicate that miR-22 is a novel target of $1,25(OH)_2D_3$ in human colon cancer cells that may contribute to explain partially the protective action of $1,25(OH)_2D_3$ on this important neoplasia.

P21-5
Epigenomic characterization of the structure-function relation in chromatin

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In eukaryotic cells DNA is packaged into chromatin, a higher order structure involving mostly nucleosomes but also a variety of non-histone proteins. Chromatin folding is a reversible and rather dynamic process regulated by ATP-dependent remodeling factors. If required, defined areas of the genome are able to decondense, thereby allowing access of DNA binding factors. Accordingly, the chromatin structure can be in general categorized as 'open' or 'closed', in which 'open' typically corresponds to transcriptionally active regions and 'closed' to heterochromatic structures [1] [2]. So far, it remained unclear how this structural classification of certain genomic loci corresponds holistically to gene regulatory functions or epigenetic modifications. As the chromatin states differ in their sensitivity to endonucleases, partial digest with micrococcal nuclease (MNase) can be used to isolate them by varying the enzyme concentration.

In this project, we apply the new method *in vivo* on native chromatin of several human cell lines, comparing e.g. health and disease or following the changes upon activation of a particular signaling pathway. Visualization of chromatin architecture by 2D and 3D fluorescence *in situ* hybridization (FISH) reveals the effective release of defined chromatin states. Comparison of the chromatin domains is carried out on a genome-wide level, applying tiling arrays and high-throughput sequencing. Thus, it is possible to identify global nucleosome positions and gain information about possibly changed nucleosome spacing. Furthermore, the identified domains can be functionally characterized by correlation analyses with genome-wide data sets regarding transcription factor and RNA-polymerase occupancies, epigenetic modifications and expression profiles. The generated data provides a perfect basis for computer simulations and predictions on a systems biological level.

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P21-6
Studies on BolA, an important protein that modulates a network of genes involved in cell growth and survival

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The *Escherichia coli bolA* morphogene is very important in adaptation to stationary phase and other stress conditions. This gene is promptly induced in harmful situations, conferring protection to the cells. Genes of this family are widespread in gram-negative bacteria and in eukaryotes. The expression of this gene is tightly regulated at transcriptional and post-transcriptional levels involving different players and mechanisms. Its overexpression is known to induce spherical morphology due to its recently described role as a transcription factor and regulator of a complex network, including penicillin binding proteins and MreB, a structural homologue of eukaryotic cell's actin. Additionally, in *E. coli*, BolA was also shown to influence biofilm formation. It is known that in nature, most bacteria live in community attached to surfaces as biofilms and these communities are of extreme relevance in biotechnology and health-related issues. Since BolA was seen to act as a transcription factor, affecting the expression of different genes of *E. coli*, we were interested in the characterization of the role of BolA in cell motility and adhesion mechanisms. We performed transcriptomic studies to obtain a global overview of BolA influence in *E. coli* transcription. We show that this protein is influencing genes with consequences in motility. Different metabolism pathways were also observed to be affected including those that are involved in the composition of the extra cellular matrix, important to promote biofilm formation. Evidence provided establishes BolA as a possible key player in the control of cell adhesion and/or motility and shows how it can modulate a network of genes involved in cell growth and survival.

P21-7
Topological stress and genome integrity maintenance

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During S-phase replication forks generate a series of topological changes known as topological stress. If not timely resolved, mounting topological stress can arrest and collapse replication forks, leading to the accumulation of DNA lesions. Topological stress generated at replication forks can also interfere with other DNA metabolic processes including gene transcription.

We investigated the replication checkpoint-dependent mechanisms controlling fork stability. This Mec1/ATR-mediated checkpoint is the major pathway promoting fork integrity following replication stress, thus counteracting mutations and chromosomal aberrations.

Factors required for gene gating, a process tethering transcribed genes to Nuclear Pore Complexes (NPCs), are phosphorylated by checkpoint kinases. We found that in response to replication stress gated genes are released form NPCs in a checkpoint-dependent manner. Nucleoporin mutations mimicking constitutive checkpoint phosphorylation were sufficient to abrogate gene gating and rescued checkpoint mutants sensitivity to the replication inhibitor hydroxyurea. Thus, checkpoint mutants' failure to release transcribed DNA from NPCs might stabilize physical barriers that accumulate topological stress consequently driving the collapse incoming forks. Consistently, ablation of gene gating-mediating factors or topological stress relaxation by induction of a double strand break between forks and gated genes counteracts fork collapse.

Our findings indicate that the replication checkpoint promotes fork stability by releasing transcribed genes association to NPCs thus allowing topological stress diffusion.

P21-8
A gene regulation mechanism allowing for the synthesis of two *ferredoxin:NADP oxidoreductase* isoforms from a single gene in the cyanobacterium *Synechocystis* sp. PCC 6803

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In photoautotrophic organisms, the main role of the Ferredoxin:NADP oxidoreductase (FNR), is to provide NADPH for CO_2 fixation. Whereas there is only one *petH* gene copy in the cyanobacterium *Synechocystis* sp. PCC6803, two FNR isoforms accumulate (FNR_L and FNR_S). It was proposed that FNR_L fulfills functions in linear electron transport while FNR_S is involved in cyclic electron transport and respiration. In addition, FNR_S was shown to be the product of an internal translation initiation within the FNR_L open-reading frame.

We discovered new aspects concerning *petH* translationinitiation regulation that leads to the accumulation of either one of the FNR isoforms. Essentially, we constructed mutants expressing ectopically FNR_L ORF under the control of either *psbAII* or *petH* 5′UTR. These mutants showed that *petH* 5′UTR was essential for FNR_S accumulation. Deletions in the 5′UTR suggested that each isoform is produced from a specific mRNA. 5′-end mapping of the *petH* transcripts confirmed this fact and showed that under standard conditions -when FNR_L accumulates- two mRNAs carrying similar leaders (32 and 53 bases) are transcribed; while under nitrogen starvation -when FNR_S accumulates, an mRNA, carrying a longer leader (126 bases), is transcribed.

EMSA showed that the global nitrogen regulator NtcA binds to the upstream region of *petH*. Mutagenesis of a putative NtcA-binding site resulted in the abolition of FNR_S accumulation. Thus we identified an NtcA bindingsite 42 nucleotides upstream from the long transcript 5′end.

Transcriptional fusion of the *E. coli lac* promoter to the *petH*-transcribed regions definitely showed that spontaneously-occurring secondary structures adopted by the longer 5′UTR activate FNR_S translation-initiation and prevent that of FNR_L.

P21-9
Regulation of PHF10 (BAF45a), the subunit of SWI/SNF chromatin remodeling complex of mammalian

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PHF10 is a transcription factor and a component of SWI/SNF chromatin remodeling complex of mam-malians and essential for transcription initiation. Previously, it was shown that PHF10 is essential for stem cells proliferation during neural development of mouse embryos [1] and immortalized human fibroblast cell lines [2]. We showed that PHF10 is ubiquitously expressed in all murine tissues. In murine tissues and human cell lines we detected endogenous PHF10 in the several forms. Based on results of phos-phatase treatment we concluded that PHF10 has a lot of post-translational modifications. We revealed three serine residues in PHF10, which are phosphorylated. We also determinated that Casein Kinase 1 (CK1) phosphorylates these amino acids. We detected another modification of PHF10 – Sumo-1.

With co-immunoprecipitation we showed that only modified form of PHF10 (endogenous and overex-pressed protein, as well) interacts with BAF200 and BAF155 (subunits of SWI/SNF complex). There are two subfamilies of SWI/SNF chromatin remodeling complexes – BAF and PBAF. With biochemical purifi-cation of PHF10-associated proteins we demonstrated that PHF10 is specific subunit of PBAF-subfamily of SWI/SNF chromatin remodeling complexes.

Multiple modifications of PHF10 can be a trigger point for activation of transcription with chromatin re-modeling complex PBAF.

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P21-10
Augmented expression of HLA-A2 surface molecules in dengue virus infection

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By presenting antigenic peptides on the membrane surface, human leukocyte antigen (HLA) molecules are critical for immune defense. Modulation of the surface expression by viruses is a major mechanism of immune escape. Conservatively, viruses down-regulate the surface expression of the HLA molecules in order to evade their eradication; however, infections with *Flaviviruses* such as West Nile Virus (WNV), Hepatitis C Virus (HCV) and Japanese Encephalitis Virus (JEV) have been associated with increased surface expression of these HLA molecules. Interestingly, this phenomenon was shown to facilitate the evasion of the

viral-infected cells from cytotoxic T Lymphocytes (CTL)-mediated cytolysis. Noteworthy, this study was designed with the aims to explore and characterize the expression of HLA–A2 molecules by Dengue Virus (DV), also a member of *Flaviviruses*. Using real-time PCR assay, evidence of the up-regulation of transcript of HLA-A2 gene was demonstrated in DV-infected cells. In addition, we proved that the up-regulation of HLA-A2 gene expression was due to the positive induction of the NFκB binding domains in the promoter region. The induction in gene expression is reflected at the level of cell-surface HLA-A2 protein as shown with flow-cytometry approach. Functional studies of the activity of the increased HLA-A2 are underway. Our current findings unravel the fundamental role of DV in modulating the MHC antigen pathway in DV-related diseases that have been posing alarming economics and social burden on affected nations, Malaysians included. This work is funded by Malaysian Minister of Science, Technology and Innovation (MOSTI); grant number 53-02-03-1073.

P21-11
Identification of interaction site between Rep20 protein and replication region of plasmid pAG20

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In many plasmid replicons is required plasmid-encoded replication initiator, Rep protein. Rep-type proteins bind to tandem directly-repeated sequences to establish the initiation nucleoprotein complex. Iteron sequence length, number and spacing between iteron repeats are typical of every replicon. Rep proteins belonging to the family replication initiators exist mainly as dimers, but only Rep protein monomers can initiate replication by binding to repetitive sequences. Rep proteins interact with the regulatory region of the DNA molecule by the secondary structure consist of intermittent alpha-helixes (HTH motif). The Rep proteins of some replicons, have an important second function, they recognize inversely-repeated sequences (operators) which overlap the promoter of their own coding genes, acting as self-repressors.

We isolated and characterized plasmid pAG20, from cells *Acetobacter aceti* CCM 3620, who for his replication encodes a small replication protein Rep20. Bioinformatic analysis, we confirmed the presence of motif with four intermittent alpha-helixes. We prepare some mutants of HTH region and identified DNA-binding site. Using S1-mapping and DNase I footpriinting methods in combination with Sanger sequencing we identify specific binding site on the regulatory region of plasmid pAG20.

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P21-12
Dynamic response to DNA damage in *S. cerevisiae*

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In this work time course expression data sets collected after a stimulus causing DNA damage were used. Three data sets had chosen from literature where the stimulus was MMS addition, UV radiation and CDC13 mutation, respectively. *S. cerevisiae* cells face to DNA damage go under a complex response mechanism. In order to reveal dynamics of this response two previously proposed approaches were used, Topological Enrichment Analysis of Functional Subnetworks (TEAFS) and NP analyses. Decrease in transcription of growth related genes and increase in that of catabolic process related genes were indicated by NP analysis from MMS addition data and the observations were affirmed by UV radiation data. In UV case additional subdivisions in general trends were also revealed. Enrichment in transcription and regulation was observed for genes at the interface of up-regulated and down-regulated modules. All observations were verified by CDC13 mutant experiments as well. Cell cycle related genes were found to be present both trends in all data sets. On the other hand, for TEAFS firstly, an integrated biological network from protein–protein interaction network, regulatory network and updated consensus metabolic network was constructed. Hereby, scoring the fluctuations of total topological properties of modules defined according to GO biological process terms, significantly responding terms were determined quantitatively. Then detailed analysis of the trends indicated by NP analysis could be done.

P21-13
Search for markers of grapes′ ripening involved in abiotic and biotic stress response, hormonal metabolism and signaling events

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Grapes (*Vitis* species) are economically the most important fruit crop worldwide. However, the complexity of molecular and biochemical events that lead to the onset of ripening of non-climacteric fruits is not fully understood which is further complicated in grapes due to seasonal and cultivar specific variance. In addition, drought, salinity, extreme temperatures and pathogens limit grapes′ production.

In order to search for markers of ripening, the mRNA expression profiles of *véraison* and mature berries were compared for three Portuguese grape varieties (Trincadeira, Touriga Nacional and Aragonês) growing in Southern Portugal during 2008 season. The variety Trincadeira that shows irregular ripening due to high susceptibility to seasonal climatic variations as well as to fungi infections was used to carry out the ripening studies both during 2007 and 2008 seasons.

Using the Affymetrix GrapeGen® genome array containing 23 096 probesets corresponding to 18 726 unique sequences we have identified a common set of 1586 modulated transcripts assigned to functional categories of 'abiotic and biotic stress response' and 'cellular homeostasis' as well as 'hormone signaling' and 'regulation of gene expression', among others. Concerning the polyamine pathway which is poorly known during ripening of grapes a more detailed analysis was carried out integrating gene expression with metabolites′ quantification. The expression of putative marker genes is currently being validated using real time qPCR. Altogether these results will give insights on the events leading to the onset of ripening but also on a common response of fruits to a combination of stresses.

P21-14
Investigating micro-RNA regulation of tetraspanins CD151 and CD9 in prostate cancer

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Rationale: Tetraspanins are small transmembrane proteins involved in the modulation of various cellular functions related to cancer progression, such as cell-cell adhesion, migration and signalling. The tetraspanin CD151 (pro-metastatic) is typically over-expressed in cancer, whereas CD9 (metastasis suppressor) is generally under-expressed in cancer. Previous studies have shown that increased CD151 expression predicts poor outcome in low-grade prostate cancer, whereas decreased CD9 expression has been shown in advanced prostate tumours and metastases. Therefore, these tetraspanins may represent useful prognostic indicators for prostate cancer. However, progress in developing CD151 and CD9 as biomarkers is hampered by a lack of understanding of the mechanisms controlling altered CD151 and CD9 protein expression in prostate cancer.
Objective: Preliminary experiments using a prostate cancer cell line series showed that CD151 and CD9 gene and protein expression levels do not correlate, suggesting CD151 and CD9 protein expression may be predominantly controlled post-transcriptionally. One post-transcriptional mechanism is control by microRNA (miRNA), small non-coding RNAs, several of which have altered expression in prostate cancer. Therefore, we aim to investigate if miRNA are able to regulate the expression of CD151 and CD9 *in vitro* using a range of prostate cancer cell lines.
Methods and Results: Using bioinformatics several miRNA known to have altered expression in prostate cancer were predicted to target CD151 and CD9. These miRNA are being assessed in a panel of prostate cancer cell lines by qPCR for expression changes which inversely correlate with their target tetraspanin expression. Selected miRNA will then be assessed for their ability to regulate CD151 or CD9 using 3′UTR luciferase reporter assays. In particular miR-25, a predicted CD9 target has shown increased expression in cancer versus normal cell lines.
Conclusion: Altered miRNA expression (e.g. up-regulation of miR-25) is one way in which prostate cancer cells might alter tetraspanin expression resulting in metastasis.

P21-15
Global effects on the distribution and activity of RNA polimerase II upon depletion of the FACT complex

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FACT is an essential component of eukaryotic cells involved in chromatin transactions. Previous studies have shown that this complex physically associates to RNA pol II and plays an important role in chromatin assembly and disassembly during the elongation step of transcription. Mutations affecting the stability of Spt16, one of the two main subunits, cause transcriptional alterations that have been mostly associated to defective nucleosome remodelling.

In order to understand the global importance of this complex in transcription, we have performed a genome-wide approach to measure the distribution of active (detected by run-on) and total (detected by chromatin immunoprecipitation) RNA pol II along the genes, in the presence or absence of the FACT complex, using a temperature sensitive allele of Spt16. Our analysis reveals that RNA pol II dependence on this complex is not homogeneous, and can relay on some physical properties of genes as length or position along the chromosome. Interestingly, FACT depletion also leads to an increase in the presence and activity of RNA pol II in many genes related with chromosome condensation and cohesion. Finally, we have found that, although a huge majority of genes show a similar behaviour in terms of activity and levels of RNA pol II upon FACT inactivation, there are certain gene groups that are differentially affected on these two parameters, suggesting that FACT plays a specific role in the control of their expression.

P21-16
New molecular insights in APE1 binding to nCARE elements of gene promoters: identification of SIRT1 as novel target gene

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The apurinic/apyrimidinic endonuclease 1 (APE1) is a multifunctional protein contributing to genome stability through its central role in BER pathway of DNA lesions, caused by oxidating and alkylating agents and playing also a role in gene expression regulation, as a redox co-activator of several transcription factors. Recently, we demonstrated a role of APE1 in RNA metabolism, opening new perspectives for this essential protein. Another interesting and yet poorly characterized function for this non-canonical DNA repair protein is associated to its ability to bind to the negative calcium responsive elements (nCaRE) of some gene promoters thus acting as a transcriptional repressor. Since nCaRE are conserved sequences located within ALU repeats, which are widespread throughout the genome, many other functional nCaRE elements could exist playing a role in transcriptional regulation of genes. We performed bioinformatic analysis for the systematic searching of functional nCaRE sequences on human genome by filtering expression profile data of genes resulting disregulated upon APE1 knockdown. Among the list of 57 genes, whose expression is potentially regulated by APE1, we focused on the human deacetylase SIRT1, due to its relevant involvement in cell stress including senescence, apoptosis, tumorigenesis and, in particular, thought to play a role in cell response to genotoxic agents through its deacetylating activity on APE1 N-domain. We showed that the human SIRT1 promoter possesses two nCaRE elements. Through a multidisciplinary approach, based on SPR, limited proteolysis, Chip and gene reporter assays, we found that APE1 N-domain is required for the stable binding of nCaRE elements and that the charged status of lysine residues K27, K31, K32 and K35 plays a central role in the regulatory function exerted by APE1 on SIRT1 gene. These findings provide preliminary evidences on the existence of an autoregulatory loop between APE1 and SIRT1 and opens new perspectives in understanding the role of nCaRE sequences on transcriptional regulation of mammalian genes.

P21-17
Deacetylation of p53 and apoptosis following genotoxic stress are regulated by sumoylation of HDAC2

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It is subject of intensive research how posttranslational modifications (PTMs) govern histone deacetylase (HDAC) dependent control of cell proliferation and apoptosis. Histone deacetylase 2 (HDAC2) is an important epigenetic regulator of gene expression and a modulator of signaling. This enzyme deacetylates ε-*N*-acetylated lysine residues of numerous proteins relevant for homeostasis. Moreover, HDAC2 has unique functions *in vivo* and its overexpression contributes to tumorigenesis. Here, we report modification with small ubiquitin-related modifier 1 (SUMO1) as a new PTM for HDAC2. In contrast to other HDACs, HDAC2 does not require sumoylation for catalytic activity. However, sumoylation of HDAC2 at lysine K462 is necessary for the binding of HDAC2 to the tumor suppressor p53 and for the deacetylation of p53 at lysine K320. This site-specific modification of p53 by HDAC2-SUMO1 strongly affects the recruitment of p53 into promoter-associated complexes. Consequently, p53-dependent expression of genes for cell cycle control and apoptosis are largely blocked in colon cancer cells expressing wild-type HDAC2. Of note, all these biological effects require HDAC2 catalytic activity and they cannot occur in cells with sumoylation-deficient HDAC2. Our findings reveal novel isoenzyme-specific molecular mechanisms and a crosstalk that links sumoylation of a deacetylase to p53-regulated cell fate decisions following genotoxic stress.

P21-18
mRNA expression levels of elastin, fibulin-5, LOXL-1, MMP-2 and MMP-9 in patients with varicose vein

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Aim: The reason of varicose vein formation is not exactly known yet; but some morphological and physiological changes of venous wall are thought to be responsible. One of those suspected reasons is the changes in quantity and content of elastin protein. There are some recent researches about elastin protein in various other diseases, but there is not enough comprehensive investigations about the mechanisms of elastin assembly in varicose vein formation. In this study, we aimed to investigate the changes in mRNA levels of elastin and some of its related proteins in formation of varicose veins.
Methods: For this purpose we used the waste variceal saphenous veins of the patients and the excess parts saphenous veins of coronary bypass patients to be the control group. mRNA levels of elastin, fibulin-5, LOXL-1, MMP-2 and MMP-9 were determined by using real-time PCR and normalized with HGPRT.
Results: We did not observed any differences in those parameters between study groups. But we found MMP-9 expressions were significantly increased in hypertensive cases in patient group. Also, Elastin and MMP-9 expressions were found to be higher in diabetic cases of patient group than the non-diabetic ones in the same group.
Conclusions: Our study was important to be evaluating such related proteins altogether in varices patients. Further comprehensive studies will be necessary to get more convincing results.
Keywords: Elastin, matrix metalloproteinases, saphenous vein, varices, expression

P21-19
PrimPol a two in one enzyme: primase and bypass polymerase

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In silico analysis predicted that human gene CCDC111 encoded a primase belonging to the AEP (Archaeo-Eukaryotic Primases). Ccdc111 was purified, and confirmed to be active as a primase and strikingly also a DNA polymerase in the presence of manganese. Construction of a null mutant in the active centre demonstrated that both activities were intrinsic to the protein (hereafter, named PrimPol).

Unlike conventional eukaryotic primases PrimPol was able to initiate nucleic acid synthesis using not only NTPs but also dNTPs with a large preference for dNTPs during the extension. The polymerase activity was also very promiscuous, as it was able to extend both DNA and RNA primers preferentially inserting dNTPs.

Remarkably, PrimPol was able to polymerize beyond some of the most common oxidative lesion in the DNA template. PrimPol could bypass abasic sites and copied 8oxoG lesions, inserting either dC (error free) or dA (error prone). Moreover PrimPol inserts 8oxodGTP in front of dA and dC with a preference for the mutagenic insertion.

In contrast to the unique primase found in eukaryotic cells (Prim1), PrimPol possesses a conserved C-terminal region containing a Zn-finger. Interestingly, deletion of the Zn-finger enhanced the polymerase activity of the protein but was detrimental for its primase activity.

Such a combination of primase and polymerase activity, together with its high capacity to tolerate oxidative damage in DNA makes PrimPol very well suited for a role in DNA maintenance. We propose that PrimPol can facilitate replication fork progress by acting as a translesion polymerase, or as a specific primase capable to reinitiate downstream of lesions that pause or block DNA elongation by replicative polymerases, particularly at the leading strand.

P21-20
Regulation of the newly discovered human Tdp2 enzyme by SUMO and Ubiquitin

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DNA topoisomerases, cellular topological problem solvers, act by producing a temporary break in the DNA molecule during virtually all chromosomal transitions. Upon the action of chemical 'topoisomerase poisons' or nearby DNA damage, this intermediate catalytic 'cleavage complex' can be stabilized, with a covalent bond between the enzyme and the nucleotide chain termini. This aberrant structure needs to be processed to assure correct repair and avoid genomic rearrangements, one of the hallmarks of tumorigenesis.

Human tyrosyl-DNA phosphodiesterase 2 (TDP2) is the only reported enzyme capable of breaking the bond between the tyrosine of the polypeptide chain and the 5′ end of the DNA molecule, typical of topoisomerase II (Top2) action. Consequently, TDP2 deficiency provokes an extreme sensibility to Top2 poisons such as Etoposide.

TDP2 has, therefore, a unique and novel enzymatic activity whose regulation and physiological role yet need to be determined. The previously reported SUMO and TDP2 interaction and that Top2 is sumoylated, polyubiquitinated and finally degraded by the 26S proteasome when trapped in cleavage complexes, suggest a reason to explore a possible TDP2 SUMO/Ubiquitin regulation mechanism in response to Top2 poison. This study explores this possible connection with a particular emphasis on the possible implications for the maintenance of genome stability.

P21r-21
PrimPol a two in one enzyme: primase and bypass polymerase

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In silico analysis predicted that human gene CCDC111 encoded a primase belonging to the AEP (Archaeo-Eukaryotic Primases). Ccdc111 was purified, and confirmed to be active as a primase and strikingly also a DNA polymerase in the presence of manganese. Construction of a null mutant in the active centre demonstrated that both activities were intrinsic to the protein (hereafter, named PrimPol).

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P21-22
Comparative analysis of two yeast Ssu72 phosphatases

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Co-transcriptional RNA processing is a highly regulated process in which the covalent modification status of the Carboxy-Terminal Domain (CTD) of the Rpb1, the major subunit of RNA polymerase II (RNAPII) plays a critical role. One of the key CTD phosphatases in eukaryotes is Ssu72. The *SSU72* gene was initially characterized in *Saccharomyces cerevisiae* as suppressor of *sua7-1* (TFIIB) defects, and it was further shown its role as a CTD phosphatase [1] and its involvement in different nuclear processes related to the coordination of transcription and 3′-end RNA processing. Homologous to Ssu72 have been characterized in *Drosophila* and Human; their enzymatic activities, compared with Ssu72 from *S. cerevisiae*, show differences in their kinetic constants of the phosphatases [2]. The *Kluyveromyces lactis* homolog to Ssu72 (KlSSu72) displays a high homology with Ssu72 including the 'signature motif' and the 'aspartate-loop' characteristic of a low molecular weight phosphatase [3]. After purifying the GST-KlSsu72 fusion protein, we performed *in vitro* analysis of the phosphatase activity using the pNPP assay. Our results show similar *in vitro* kinetic values of the two yeast phosphatases. The KlSsu72 phosphatase activity is blocked by the oxianion inhibitor Vanadate. Expressing KlSsu72 in *S. cerevisiae*, the CTD serine 5 dephosphorylation is defective. Therefore, the differences *in vivo* suggest differences in the molecular machineries operating in the modulation of the CTD code in yeast.

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P21-23
Silencing of toxic gene expression by Fis

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Bacteria and bacteriophages have evolved DNA modification as a strategy to protect their genomes. A novel DNA modification (N^6-(1-acetamido)-adenine), a function of phage Mu *mom* gene renders Mu genome refractile to numerous restriction enzymes and confers on the phage a wide bacterial host range. However, *mom* expression is cytotoxic to the host. A strong fortification, a combined activity of the phage and host factors prevents untimely expression of *mom* and associated toxic effects. Here, we identify the bacterial chromatin architectural protein Fis as an additional player in this crowded regulatory cascade[1]. Both *in vivo* and *in vitro* studies indicate that Fis acts as a transcriptional repressor of *mom* promoter. Furthermore, Fis mediates its repressive effect by denying access to RNA polymerase at *mom* promoter. We propose that a combined repressive effect of Fis and previously characterized negative regulatory factors keeps the gene silenced most of the time. In the late lytic stage of the phage life cycle when *mom* function is required, Fis mediated repression is overcome by the transcriptional activator Mu C. We thus present a hitherto unknown facet of Fis function in Mu biology. In addition to bringing about overall downregulation of Mu genome, Fis also ensures silencing of the advantageous but potentially lethal *mom* gene. Silencing of horizontally transferred genes in bacterial genomes is a growing area, with increasing number of host protein/RNA molecules being reported to protect bacteria from the toxic effects of laterally acquired genes or genomes. Thus, Fis joins the league of proteins which in addition to their canonical cellular functions, participate in the silencing of xenogeneic DNA.

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P21-24
Epigenetics at replication factories in embryonic stem cells

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DNA replication is a fundamental process for all forms of life. The basic machinery involved in the replication of bacterial genome is conserved until mammals. However, how the integrity of the complex eukaryotic genomes is transmitted in each generation and how the replication co-exists with other chromatin activities remain elusive. To shine lights on these questions, here we use an unbiased large-scale proteomic strategy to identify proteins specifically enriched in nascent DNA during the replication of embryonic stem cells (ESCs). Our list of components reveals unexpected functions and directly links the DNA replication with the cell cycle control, the cell metabolism, the genomic stability and the control of self-renewal in ESCs. Moreover, we provide evidences for the formation of a macromolecular complex acting as a vector for epigenetic memory. Our study supposes a proteomic reference in ESCs and can orients target-directed strategies on DNA replication and epigenetic inheritance research.

P21-25
Sequence-specific epigenetic variation in the TNS3 gene promoter and its relation to Tensin3 expression in human kidney cancer

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Tensin3 is an intracellular protein that inhibits cell motility through regulation of cytoskeletal dynamics. In cancer, therefore, loss of Tensin3 may remove a suppressive effect on cancer cell motility and thereby facilitate metastasis. We have previously reported that Tensin3 expression is downregulated in human renal cell carcinoma (RCC). We therefore speculate whether epigenetic mechanisms might account for this downregulation, via e.g. *TNS3* gene promoter hypermethylation. The aims of this study are to (i) investigate the putative promoter activity of a CpG island close to the *TNS3* gene and (ii) determine whether this region is alternatively methylated in human RCC.

Bioinformatic analysis identified a putative promoter in the human *TNS3* gene, which contained an 826bp CpG island. Varying size fragments of this region were cloned into a promoterless firefly luciferase vector and subsequently transfected into kidney HK2 cells for luciferase reporter analysis. Additionally, genomic DNA from human kidney tissues (matched tumour and non-tumour adjacent) was bisulphite converted for PCR to amplify specific segments of the *TNS3* CpG island; these segments were subsequently analysed by pyrosequencing for quantitation of CpG methylation. Concurrently, Tensin3 expression in RCC cells was analysed by qRT-PCR following treatment with the demethylating drug 5-aza-2′deoxycytidine.

Initial analyses indicate a functional promoter activity in the *TNS3* island region identified. We have quantitatively determined the methylation level of 43 CpG dinucleotides in this region. There was a threefold increase in overall methylation levels in RCC tumour samples (tumour $n = 342$, adjacent $n = 87$; $p > 0.001$). The majority of CpG dinucleotides were unchanged in their methylation levels, whereas a number of specific CpG dinucleotides showed higher methylation levels in tumour samples. Furthermore, demethylation treatment of HK2 cells lead to a threefold upregulation of Tensin3 expression. In conclusion, these results may provide new insights into the epigenetic basis for downregulation of Tensin3 in kidney cancer.

P21-26
RNA-Seq analysis of the response to nitrogen stress in a filamentous cyanobacterium. Identification of non-coding RNAs

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Cyanobacteria are a group of photosynthetic organisms that are not only responsible for a significant proportion of CO2 fixation on Earth but also in many cases able to fix atmospheric nitrogen. Because nitrogenase, the enzyme that catalyzes nitrogen fixation, is extremely sensitive to oxygen, nitrogen-fixing cyanobacteria have developed sophisticated strategies to avoid inhibition of nitrogenase by oxygen. In filamentous strains, one such strategy is the differentiation of specific cells, the heterocysts, that appear regularly spaced along the filaments and are devoted to nitrogen fixation. Differentiation of heterocysts involves a strictly regulated transcriptional program that is initiated in response to nitrogen deficiency and transforms an oxygen-evolving photosynthetic cell into a non-photosynthetic heterocyst, which establishes precise relationships with vegetative cells.

Non-coding RNAs (ncRNAs) have been found to be involved in the regulation of virtually every bacterial response to stress. We have begun a global approach to the identification of ncRNAs in heterocystous cyanobacteria. Our approach is based on a transcriptomic analysis carried out by deep sequencing of total RNA (RNA-Seq) isolated from cells that are subjected to nitrogen deficiency for 8 h (in comparison to control samples not subjected to nitrogen deficiency). We have identified several putative non-coding RNAs that exhibit a strong regulation of their expression in response to nitrogen availability, and therefore might correspond to functional non-coding RNAs involved in the regulation of the responses to nitrogen stress and/or heterocyst differentiation.

P21-27
Acidic pH increases phosphatidylserine-dependent phagocytosis in macrophages via upregulation of stabilin-1 expression

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Microenvironmental acidosis is a common feature of inflammatory loci, in which clearance of apoptotic cells is necessary for the resolution of inflammation. Although it is known that a low pH environment affects immune function, its effect on the phagocytic activity of macrophages remains to be investigated. Here, we show that extracellular low pH enhanced the phagocytic capacity of macrophages by increasing expression of stabilin-1, which is involved in PS-dependent removal of apoptotic cells. Low pH selectively increased stabilin-1 expression at both the mRNA and protein levels in macrophages. Blockade of stabilin-1 in macrophages abolished the enhancement of phagocytic activity by low pH. Reporter assays showed that the -120/-1 region of the mouse stabilin-1 promoter was a low pH-responsive region and provided evidence that extracellular low pH mediated transcriptional activation of stabilin-1 via Ets-2. Furthermore, extra-

cellular low pH activated c-Jun N-terminal kinase (JNK), thereby inducing translocation of Ets-2 into the nucleus. Thus, our results suggest that a low pH microenvironment at inflammatory loci can modulate the phagocytic capacity of macrophages and establish roles for stabilin-1 and Ets-2 in the maintenance of tissue homeostasis by the immune system.

P21-28
Positioning and dynamic of Nucleosome N + 1 in *Jmjd6, Sap30l* and *Suv39h2* genes

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Nucleosomal dynamics is essential in regulating gene expression and other processes in eukaryotes. These should be moved and/or partially unstructured to allow transcription and DNA replication. The nucleosome N + 1 in particular, occupies different positions in the state of activation and genes repression. The stability of nucleosomes depends mainly on the histone code and chromatin remodelling.

The *Jmjd6*, *Suv39h2* and *Sap30I* encode proteins involved in the regulation of chromatin fuction. The protein encoded by *Sap30I* is a subunit of the Sin3-histone deacetylase complex, *Jmjd6* encodes a histone arginine demethylase, which is capable to demethylate H3R2 and H4R3 histone residues, whereas *Suv39h2* encodes a histone methyltransferase that specifically trimethylates H3K9 residue. It is expected that the dynamics of nucleosome N + 1 has an important role in the expression of these genes and should be highly regulated in the celular response in several diseases such as cancer. However, little is known about nucleosomal positioning of these genes and how the dynamic changes may be regulated by specific histone modification marks.

The partial hepatectomy (PH) is a excellent model to study the dynamic of nucleosome positioning since liver regeneration activate a massive and synchronized cell division triggered by the expression of cell cycle regulating genes. This work uses the model to study changes in the positioning of nucleosomes in these chromatin-regulating genes carried out from mononucleosomal obtained by digestion with micrococcal nuclease. The mononucleosomal-size DNA fragments were analyzed using qPCR with overlapping amplicons in the start of the transcribable región of each gene. The results allowed to identify the region where the N + 1 nucleosome is located and described the dynamic changes in its position, as a result of transcription of the *Jmjd6*, *Sap30I* and *Suv39h2*, during initiation of hepatocyte division.

P21-29
Multiple levels of GCN2 inhibition by HIV-1 proteins

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The reversible phosphorylation of the α-subunit of eukaryotic translation initiation factor 2 (eIF2α) is a well-characterized mechanism of translational control in response to a wide variety of cellular stresses, including viral infection. Beside PKR, the eIF2alpha kinase GCN2 participates in the cellular response against viral infection by RNA viruses with central nervous system tropism. PKR has been involved in the antiviral response against HIV-1, although this antiviral effect is very limited due to the distinct mechanisms evolved by the virus to counteract PKR action. Here we report that infection of human cells with HIV-1 conveys the proteolytic cleavage of GCN2 and that purified HIV-1 and HIV-2 proteases produce direct proteolysis of GCN2 *in vitro*, abrogating the activation of GCN2 by HIV-1 RNA. Moreover, the HIV-1 protein Tat interacts with PKR and inhibits its *in vitro* activity by acting as a competitive inhibitor due to binding to the same site of eIF2α on the kinase. We have assayed *in vitro* eIF2α kinase activity of GCN2 and several GCN2 mutants in the presence of Tat, and we have also observed a reduction of eIF2α phosphorylation in all cases. These significant findings suggest that cleavage of GCN2 by HIV-1 protease and competitive inhibition by Tat could represent distinct mechanisms of HIV-1 to counteract GCN2 antiviral activity.

P21r-30
Dioxin receptor transcription factor binding to Alu elements X14S, X36S and X45S modulates the expression of stemness-relevant genes Oct4, Nanog, Shh, Sox2 and KLF4

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Eukaryotic genomes are organized into expression domains whose activity depends on local chromatin structure. To accomplish that, functional gene clusters are defined by the binding of transcription factors to conserved regulatory sequences. Transposable elements are no longer considered parasite mobile sequences; instead they seem to regulate gene expression by modulating genome organization and stability. Such transposon functions are likely associated to their ability to bind specific transcription factors. We have previously reported that a genome-wide SINE-B1 retrotransposon (B1-X35S) has a potent insulation activity by binding of the transcription factors dioxin receptor (AhR) and Slug (Snai2) to their consensus elements present in the transposon sequence. In the human genome, three Alu elements have been identified that are heterologous to the B1-X35S mouse transposon and that contains a conserved AhR binding site. Interestingly, these Alus (hereafter X14S, X36S and X45S) are present in most stemness-relevant genes including Oct4, Nanog, Shh, Sox2 and Klf4. In the undifferentiated embryonic carcinoma Ntera-2 cell line, treatment with retinoic acid (RA) induces differentiation by decreasing Oct4, Nanog and Shh expression levels. Remarkably, RA-induced differentiation promotes a parallel increase in AhR protein. Silencing of AhR by RNA interference (siRNA of shRNA) blocks the decrease in Oct4, Nanog and Shh induced by RA thus maintaining an undifferentiated state. Chromatin inmunoprecipitation (ChIP) experiments were performed to confirm AhR binding to the X14S, X36S and X45S Alu elements present in the promoter of such target genes. In addition, enhancer blocking assays (EBAs) will be used to address the insulator activity of X14S, X36S and X45S Alu elements. Their insulator activity will be confirmed *in vivo* in zebrafish. We suggest that X14S, X36S and X45S Alu elements can represent evolutionary conserved genome-wide insulators activated by the transcription factor AhR to control developmental, oncogenic or toxicological-dependent processes.

P21-31
Regulation of drugs metabolizing enzymes and transporters in mouse liber by primaquine

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Malaria is one of the greatest of all infectious diseases, afflicting more than 500 million people and causing around 2 million deaths each year.

Although many classes of drugs have been used in the treatment and prophylaxis of malaria, therapeutic failure is an increasingly important problem either through parasite resistance or variability in the host's metabolism.

Drug metabolizing enzymes (mainly Cytochromes P450) and transporters are often involved in therapeutic efficacy as well as in clinically significant drug-drug interactions. An additional source of variability is the regulation of those genes, which is mediated by a group of nuclear receptors.

Although the genes involved in mammalian drug metabolism and transport have been object of many studies, their response to antimalarial drugs is poorly understood.

In this study we evaluated the effect of antimalarial drugs in the hepatic expression levels of these genes using a rodent malaria model. Expression patterns were evaluated by RT-PCR in three strains of mice infected with *Plasmodium chabaudi* and subject to a treatment with primaquine. The analysed genes included Cytochromes P450 (from families *Cyp1*, *Cyp2 and Cyp3*), ABC transporters (*abcb1*) and nuclear receptors (PXR and CAR).

The observed changes in gene expression triggered by primaquine exposure may be relevant for the understanding of the mechanisms underlying drug failure and drug-drug interactions, two of the most serious problems in malaria therapeutics.

P21-32
New patterns of antizyme inhibitor 2 expression in secretory tissues

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Polyamines play an important role in cell growth and differentiation, and although polyamines have been found in secretory vesicles, their function in secretory processes is mostly unknown. We report here the expression of antizyme inhibitor 2 (AZIN2), a positive regulator of polyamine biosynthesis and transport, which was recently characterized by our group, in different secretory cells and tissues. We used qRT-PCR analysis of AZIN2 mRNA of wild type mice as well as the expression analysis of the reporter gene beta-galactosidase in transgenic mice, generated by using the gene trap technology, which expressed transgenic β-galactosidase under the control of AZIN2 endogenous promoter. AZIN2 was expressed not only in testis and brain, as previously reported, but also in secretory tissues such as pancreas and the adrenal glands. Interestingly, biochemical and histochemical analyses of these tissues of transgenic mice revealed that AZIN2 was mainly expressed in the chromaffin cells of the adrenal medulla and in the Langerhans islets of pancreas. In these cells, β-galactosidase staining was found in the cytosol and in subcellular granules. Co-localization experiments carried out by immunofluorescence analysis showed that all β-gal positive cells in the pancreatic islets were insulin producer β cells, although not the entire population came to show AZIN2 expression at all. These results, together with the subcellular localization of AZIN2 in the Golgi network and the recently reported expression of AZIN2 in mastocytes, suggest that AZIN2 may have a role in the modulation of secretory cells, by affecting the polyamine uptake by the secretory granules and the vesicular trafficking.

P21-33
Cladosporol A inhibits HT-29 cell proliferation through inactivation of the β-catenin/TCF pathway mediated by a PPARγ–dependent mechanism

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Cladosporol A, a secondary metabolite from *Cladosporium tenuissimum*, has recently been shown to exhibit antiproliferative properties in various human colon cancer derived cell lines through modulation of gene expression of several cell cycle gatekeepers. Specifically, we demonstrated that cladosporol A induces inhibition of cell proliferation by the upregulation of $p21^{waf1/cip1}$ gene expression mediated by an Sp1-dependent, p53-independent mechanism. To obtain these effects, a functional PPARg is required, indicating that the drug acts as a natural ligand of the receptor. In this work, we report that exposure of HT-29 cells to cladosporol A causes reduced expression and nuclear distribution of b-catenin, a key molecule involved in the carcinogenesis of various tissues, including colon. This result well correlates with a decrease of c-MYC and cyclin D1, two recognized targets of the b-catenin/TCF pathway and a simultaneous increase of E-cadherin expression. E-cadherin is involved in cell-cell and cell-extracellular matrix interactions and is a well-recognized gene target of PPARg because a PPRE motif is contained in its promoter region. On the basis of these results, we propose that, acting as a new PPARg-ligand, cladosporol A inhibits cell migration and hence the metastatic potential, and inhibits cell proliferation through regulation of $p21^{waf1/cip1}$, cyclin D1, cyclin E, CDK2, CDK4 expression. On the other hand, it inhibits the b-catenin/TCF pathway and transcription of its target genes, among which c-*myc* oncogene.

P21-34
Replication slippage induced by the Uup protein from *Escherichia coli*

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Replication slippage errors are mainly associated to DNA repeated sequences able to adopt secondary structures. We have previously demonstrated that hairpin-prone sequences block polymerase progression *in vitro*, leading to polymerase dissociation and DNA template/primer misalignment, generating thus, deletions on the DNA (Viguera et al, 2001a, b).

We have performed a structural and genetic study of the Uup protein from *E. coli*. This protein belongs to the ABC family encoding an ATP-binding cassette ATPase. ABC systems belong to a large superfamily of proteins that couple the energy released

from ATP hydrolysis to a wide variety of cellular processes, such as molecule transport, gene regulation and DNA repair. Inactivation of *uup* leads to an increase in the frequency of Tn*10* and Tn*5* transposons excision and long tandem repeat deletions in *E. coli*. Precise excision of transposons is independent of transposase and RecA homologous recombination and it is thought to occur by a replication slippage event between direct repeats. On the other hand, tandem repeat deletions is characteristic of DNA replication mutants. Altogether, a role of the Uup protein in DNA metabolism has been suggested.

The *uup* gene encodes a 72 kDa polypeptide able to bind DNA *in vitro* in a non-sequence specific manner (Murat et al. 2006), so the effect of this protein on transposon excision have been proposed to be mediated by the direct binding to DNA. Uup contains two conserved ATP-binding domains separated by a 75-residue linker, and a C-terminal domain (CTD). Deletion of the CTD domain abolishes Uup function and reduces DNA-binding by twofold (Burgos-Zepeda, 2010). Furthermore, the isolated CTD domain is able to bind DNA with a tenfold lower affinity than intact Uup, contributing thus to the DNA binding ability of Uup.

In order to better understand the role of Uup in DNA replication, its ability to bind to DNA structures that mimic replication fork intermediates has been analysed. Furthermore, viability of *uup* double mutants with several replication genes will be presented.

P21r-35
Mesencephalic astrocyte-derived neurotrophic factor from Drosophila melanogaster is both a structural and a functional ortholog of human Manf gene

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Previously we showed that *DmManf* is an important gene for the normal development and essential for survival. Continuing exploring the functions of *DmManf* gene, the only structural ortholog of human *Manf* in flies, herein we present the rescue phenotype results for the most informative *DmManf loss-of-function* mutants obtained. The homozygotes mutants carrying the $Manf^{delta1151}$and $Manf^{delta301}$ different alleles have revealed polyphasic stages of lethality, from embryos to third instar larvae. Gene therapy experiments using ActinGAL4 specific driver inducing both *DmManf* and humans' *Manf* cDNA transgenes confirm the *DmManf* unequivocally involvement in the lethality of the *DmManf* homozygous deletions mutants and the supposed functional orthology between Drosophila and human *Manf*. The cumulative percentage of the rescued transgenic adult flies was about 57%, comparing with 100% which indicates the maximum expected value of complete rescue estimated by *Mendelian* inheritance. These experiments allowed us to infer that *DmManf* and human *Manf* could share the yet unknown cognate receptor, important for a normal development and essential for viability. The study gains importance not only for *Drosophila*, but also for humans, especially due to the fact that human *Manf* was also frequently found to be associated with various solid cancers. New directions of research for comparative studies of these genes/proteins have just been started, giving potential insights to treat human pathologies associated with human *Manf* mutations.

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P21-36
Computational identification of transcriptionally co-regulated genes, validation with the four ANT isoform genes

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The analysis of gene promoters is essential to understand the mechanisms of transcriptional regulation required under the effects of physiological processes, nutritional intake or pathologies. In higher eukaryotes, transcriptional regulation implies the recruitment of a set of regulatory proteins that bind on combinations of nucleotide motifs. We developed a computational analysis of promoter nucleotide sequences to identify co-regulated genes by combining several programs that allowed us to build regulatory models and perform a crossed analysis on several databases. This strategy was tested on a set of four human genes encoding isoforms 1–4 of the mitochondrial ADP/ATP carrier ANT. Each isoform has a specific tissue expression profile linked to its role in cellular bioenergetics. From their promoter sequence and from the phylogenetic evolution of these ANT genes in mammals, we constructed combinations of specific regulatory elements. These models were screened using the full human genome and databases of promoter sequences from human and several other mammalian species. For each of transcriptionally regulated ANT1, 2 and 4 genes, a set of co-regulated genes was identified and their over-expression was verified in microarray databases. Most of the identified genes encode proteins with a cellular function and specificity in agreement with those of the corresponding ANT isoform. The tissue specific gene expression is mainly driven by promoter regulatory sequences located up to about a thousand base pairs upstream the transcription start site. This study should provide, along with transcriptomics and metabolomics, assistance in developing cellular metabolic networks and their regulatory pathways.

P21-37
Characterization of Nrl1 in *S. pombe*: a link between RNAi and poll regulation?

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Recent evidence indicates the existence of a physical and functional link between RNA-interference (RNAi) and RNA polymerase II (Pol II) throughout the eukaryotic kingdom. However, the role of this complex partnership on the metazoan heterochromatin landscape remains still unexplored. In *Caenorhabditis elegans*, the NRDE-2 protein connects RNAi and Pol II inhibition, in a process of co-transcriptional gene silencing (CTGS). By in silico analysis, I have identified the ortholog of *C. elegans* NRDE-2 in *S. pombe*, and I have named it NRde-2 Like protein 1 or Nrl1. My research focuses on investigating whether and how Nrl1 may also represent a link between RNAi and Pol II in *S. pombe*. Since Nrl1 has orthologs also in Arabidopsis, Drosophila and human this study may produce important contributions in the field of metazoan RNAi-mediated CTGS, going far beyond the specific yeast system I will use in this work.

P21-38
Genome-wide study of chromatin remodeling factor CHD8 role in transcription

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CHD8 (Chromodomain-Helicase-DNA binding protein 8) is a member of the chromodomain helicase DNA-binding (CHD) subfamily of enzymes, which also belongs to the SNF2 family of ATP-dependent chromatin remodelers. Previous studies of our group showed that depletion of CHD8 impairs cell proliferation. We also demonstrated that CHD8 controls the expression of cyclin E2 (CCNE2) and thymidylate synthetase (TYMS), two genes expressed in the G1/S transition of the cell cycle. In order to identify CHD8 target genes, in the present study we performed a ChIP-on-chip genome-wide analysis. The results show that CHD8 binds to over 1900 genes and its recruitment is preferentially located at transcription start site (TSS), first exon and first intron. Remarkably, ChIP-on-chip results show a great correlation between CHD8 occupancy and the presence of histone H3 di- and trimethylated at lysine 4 (H3K4me2 and H3K4me3) activation marks, suggesting that CHD8 is mainly involved in transcription activation. Interestingly, the analysis of annotated transcription factor binding sites in DNA sequence indicates that a high number of CHD8 target genes are also regulated by E2F, a transcription factor involved in cell cycle regulation. Remarkably, we can see by ChIP analysis that CHD8 is recruited to the promoters of a subset of E2F target genes in exponentially growing RPE1 cells. Moreover, we observe by RT-qPCR analysis that in CHD8-silenced cells transcription regulation is impaired. Ongoing studies will address the functional regulation and implications of CHD8 in coordination with E2F to regulate target genes and cell cycle.

P21-39
Rtp1p is a karyopherin-like protein involved in the nuclear import of RNA polymerase II in yeast

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The structure and functional regulation of RNA pol II have been well characterized, but only recently the study of its biogenesis has been undertaken (reviewed in 1).

In a previous work [2], we isolated mutants that suppress the growth defect caused by the depletion of NC2. We identified mutations affecting components of three different complexes involved in the control of basal transcription: the mediator, TFIIH and RNA pol II itself. Besides, we isolated mutations in *IWR1* and a previously uncharacterized gene, which we named RTP1. Recently, Cramer and col. reported that Iwr1p directs the nuclear import of RNA pol II [3]. Here, we report the characterization of the *RTP1* gene.

The structure models obtained from the I-TASSER server show a HEAT-repeat architecture for Rtp1p, characteristic of karyopherins. In agreement with its predicted structure, Rtp1p interacts *in vivo* and *in vitro* with FG-containing nucleoporins Nup100p and Nup116p. We found that Rtp1p is required for the nuclear localization of RNA pol II, but not for those proteins containing a classical nuclear localization signal, which are imported by the importin a-dependent pathway, such as Iwr1p. Finally, we observed physical interactions between Rtp1p and several subunits of RNA pol II, as well as components of the RTP2 complex, which has been involved in RNA pol II biogenesis [4]. Taken together, our results suggest that Rpt1p participates in the assembly and/or nuclear import of RNA pol II.

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P21-40
Inhibition of murine DNA methyltransferase Dnmt3a by dimeric bisbenzimidazoles and mechanism-based inhibitors

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The DNA methyltransferase Dnmt3a is overexpressed in various types of tumors, suggesting its involvement and important role in tumorigenesis. Dnmt3a depletion significantly inhibits tumor cell growth due to reactivation of epigenetically silenced tumor suppressor genes. Therefore, Dnmt3a inhibitors may serve as antitumor drugs. We studied the inhibition of a catalytic domain of murine Dnmt3a (Dnmt3a-CD) by a series of novel non-nucleoside inhibitors, dimeric benzimidazoles DB(n), and by a mechanism-based inhibitor 2-(1H)-pyrimidinone (P).

DB(n) are DNA minor groove ligands differing in the length of methylene linkers joining bisbenzimidazole fragments. DB(n) were found to inhibit Dnmt3a-CD with an IC_{50} of 5–77 μM. The lowest IC50 value was observed for the compound DB(11) with an 11-unit methylene linker. Increasing the time of DNA incubation with DB(n) and addition of AT clusters to DNA enhanced the inhibitory effect. The observed inhibition by DB(n) may result from interference of the bulky substituent with the interaction of Dnmt3a catalytic loop with DNA minor groove. We showed that the use of DB(n) as MTase inhibitors *in vivo* is promising due to their capacity to penetrate through cell and nuclear membranes and due to low citotoxicity. Treatment of lung embryonic fibroblasts F-977 with DB(3) led to reduction of methylation of 18S RNA gene.

DNA duplexes containing P instead of the target cytosine inhibited Dnmt3a-CD due to formation of a covalent intermediate that was resistant to 0.1% SDS. The conjugates of Dnmt3a-CD with P-DNA did not withstand heating at 65° unlike M.HhaI- (P-DNA) conjugates. The yield of the covalent intermediate decreases in the presence of the minor groove ligand DB(11), indicating the importance of DNA minor groove for covalent intermediate formation. The inhibition by P-containing DNA duplexes was competitive with respect to substrate, and IC50 value was equal to 830 nM. We speculate that 2(1H)-pyrimidinone-ribonucleoside (zebularine), a similar compound and a promising antitumor drug, inhibits Dnmt3a *in vivo*.

P21r-41
New insights into the function of THO/TREX mRNP biogenesis and export factor in yeast and human cells

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Transcription is a central function occurring in the nucleus of eukaryotic cells in coordination with other nuclear processes. During transcription, the nascent pre-mRNA associates with mRNA-binding proteins and undergoes a series of processing steps, resulting in export-competent mRNA ribonucleoprotein complexes (mRNP) that are transported into the cytoplasm. THO is a eukaryotic conserved complex that acts at the interface between transcription and mRNP export. The yeast THO complex is formed by four strongly interacting subunits (Tho2, Hpr1, Mft1 and Thp2) and Tex1. THO complex associates with mRNA export factor Sub2/UAP56 and Yra1/Aly in a larger structure called TREX. In yeast, THO/TREX mutations lead to transcription elongation defects, mRNA export defects and an increase in genetic instability, measured as an increase of transcription-associated recombination. Recently, we have shown that human THO/TREX depletion leads to a similar phenotype in human cells, consistent with an evolutionary conservation of the functional connection between these mRNP biogenesis factors and genome integrity (Dominguez-Sanchez et al., PLoS Genetics 2011). Despite the conservation of THO/TREX it is unclear whether the functional relevance of all subunits is the same in all eukaryotes. Here we carried out the analysis of the effect of the heterologous expression of UAP56, the human ortholog of Sub2, in yeast. We have observed that the expression of hUAP56 partially suppresses the thermosensitivity phenotype and transcription defects of yeast *sub2* mutants. In order to get more insight in the role of the different subunits of human THO/TREX we are performing two-hybrid screenings to identify new protein partners that could transiently interact with THO/TREX. The results will be discussed.

P21-42
Identification of the LMN-1 and EMR-1 DNA binding sites reveal the genome organization at the nuclear envelope in *C. elegans*

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Besides its role as a barrier between the nucleus and the cytoplasm, in the last years it has been revealed as a dynamic structure that plays an important role in the regulation of gene expression and chromatin organization. Traditionally, the NE has been associated with heterochromatin and silent DNA. However, recent studies have shown that there is also active chromatin at the nuclear periphery and genes that change their localization depending on their transcriptional state. Despite the efforts realized, the molecular mechanisms underlining all these processes are not well understood. Here we have developed tools to perform genome wide analysis using the DamID and RNA-seq methods. DamID is based on the expression *in vivo* of chimeric proteins containing an adenine methyltransferase (Dam) from *E. coli* that methylates the DNA in the vicinity of native binding sites of a chromatin-interacting protein. We have created *Caenorhabditis elegans* strains containing single copy insertions of Dam fused to two NE proteins, emerin/EMR-1 and lamin/LMN-1. Employing a genetically amenable model system enables us to analyze the nuclear architecture across several NE mutant backgrounds, and trough different developmental stages. Besides, we have performed RNA-seq analysis in wild type and NE mutants to correlate the position of genes with their transcriptional state.

P21r-43
Functional implication of SREBP2 nuclear factor in the SNDp102 gene transcription

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Staphyloccocal Nuclease and Tudor domain containing protein of 102 kDa (SNDp102) is a protein encoded by the rat Snd1 gene. SND1, the homologue protein of rat SNDp102, first identified as a transcriptional coactivator, is a multifunctional protein with a role in siRNA and mRNA posttranscriptional processing. The presence of SND1 in non-nuclear regions of lipid secretory tissues and lipid droplets suggests a role for the protein in lipid metabolism. Indeed, the overexpression of SND p102 in rat hepatocytes, promotes the secretion of phospholipid-rich lipoproteins and it has been recently associated to lipid bodies in a hepatocellular steatosis cell model. Sterol regulatory element-binding protein 2 (SREBP2) is a transcription factor mainly involved in regulation of cholesterol biosynthesis. Bioinformatic analysis of the SNDp102 promoter sequence (AY957585) predicted multiple putative binding sites for SREBPs. To elucidate the role of SREBP2 in the SNDp102 gene transcription, 5′deletion fragments of the gene promoter and the SREBP-2 expression vector were co-transfected in rat McA-RH7777 cells and the transcriptional activity determined. A reduction in luciferase activity (45–60%) was observed in fragment 422 and longer ones. By Chromatin Immunoprecipitation assays using specific antibodies against SREBP2 we demonstrated the union of the transcription factor to a SNDp102 promoter region containing some binding sites. Ours results suggest an inhibitory role of SREBP in the control of SNDp102 expression, without discarding the involvement of additional transcription factors in a more complex regulatory mechanism.

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P21-44
Regulation of transcription in response to heat stress

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All cells, whether unicellular or part of a multicellular organism, must coexist with a variety of environmental fluctuations that might be harmful or even lethal. One of the most frequent environmental variations is the increase of temperature that leads to several changes in cells known as the heat stress response which includes protein denaturalization, transient cell cycle arrest and variations in membrane fluidity and structure. Consequently, the ability to rapidly respond to temperature fluctuations is essential for cell viability. Cells exposed to environmental stress alter the transcriptional program, resulting in the immediate down-regulation of housekeeping genes and the increase of defense and/or adaptation genes. Defense response to heat stress is characterized by a rapid induction of genes that encode for Heat Shock Proteins (Hsp), which keep protein homeostasis, relieving the folding defects and preventing protein aggregation and cellular damage. In order to define the global cellular adaptation response to heat

stress, we performed a systematic genetic screen that yielded 277 yeast genes required for growth at high temperature. We found that the Rpd3 histone deacetylase complex was enriched. Global gene expression analysis showed that Rpd3 partially regulated gene expression upon heat shock. The Hsf1 and Msn2/4 transcription factors are the main regulators of gene activation in response to heat stress. *RPD3*-deficient cells had impaired activation of Msn2/4-dependent genes, while activation of genes controlled by Hsf1 was deacetylase-independent. Rpd3 bound to heat stress-dependent promoters through the Msn2/4 transcription factors, allowing entry of RNA Pol II and activation of transcription upon stress. Finally, we found that the large, but not the small Rpd3 complex regulated cell adaptation in response to heat stress.

P21-45
Transcriptional regulation by the stress-activated protein kinase p38

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Exposure to stress requires rapid and efficient adaptive responses to maximize cell survival. Cellular stress results in activation of a conserved family of Stress Activated Protein Kinases (SAPKs), which include the mammalian p38 and the yeast Hog1. Regulation of transcription by SAPKs is an essential aspect for adaptation to extracellular stimuli. We have seen that the p38 SAPK plays a central role in gene expression regulation upon stress, as up to 80% of the up-regulated genes upon stress are p38 SAPK dependent. In mammals, the activation of p38 results in the regulation of gene expression through the direct phosphorylation of several transcription factors. In addition, p38 is recruited to stress-induced promoters via its interaction with transcription factors to proper regulate gene transcription. Once recruited, p38 activity allows the binding of RNA Pol II to stress-responsive promoters. On the other hand, the presence of active p38 at stress-responsive open reading frames also suggests the involvement of the SAPK in transcriptional elongation. Altogether, these results establish the p38 SAPK as an essential regulator in the transcriptional response to stress.

P21-46
Deficiency of chromosomal initiation of replication suppress thymineless death in *Escherichia coli*

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Cells requiring thymine for growth experience lethality when they are starved for thymine, this phenomenon experienced by prokaryotic and eukaryotic cells is defined as thymineless death (TLD) and it is prevented by rifampicin addition. We obtained evidences indicating that, among the effects of the inhibition of RNA polymerase, the rifampicin-sensitive step of chromosomal initiation is the target related to TLD suppression (Mata & Guzmán 2011, *DNA Repair* 10: 94–101). We studied whether altering the required RNA transcription level at the origin region would impair the chromosomal initiation capacity (ChIC) under thymine starvation, alleviating TLD. By using mutation in genes surrounding *oriC* chromosomal origin we show that abolishing (in *mioC112* and *gid113* mutant strains) or increasing (in *mioCp9* mutant strain) the required transcription level around *oriC* alleviates TLD by limiting the initiation capacity generated under thymine starvation. Furthermore, we found that TLD is suppressed in *oriC* deletion strains. We show that in these genetic backgrounds the efficiency of initiation of chromosomal replication was drastically reduced and ChIC was almost abolished. This effect was clear-cut when the deleted region contained the left half of the replication origin or the DnaA-boxes located on the right half of the *oriC* region. These results indicate that initiation of chromosome replication is a critical target for the lethal injuries under thymine starvation concluding in TLD. The relationship between cell death and the initiation events show them as new therapeutic target for the treatments based on the inhibition of thymine metabolism; opening the possibility to develop new therapies improving the lethal effect on microbes and cancerous cell.

P21-47
RedR1 and RedR2, two NtrC-family regulators, control the resorcinol (1,3 dihydroxybenzene) anaerobic degradation pathway in Azoarcus anaerobius

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Resorcinol (1,3-dihydroxybenzene) can be used as the sole carbon and energy source by Azoarcus anaerobius a strictly anaerobic nitrate reducing bacteria. The pathway involves two consecutive oxygen-independent oxidative reactions [1]. The genes for the degradation pathway are located in a chromosomal region of 29 kb which includes 19 genes [2]. RT-PCR analysis showed that the genes of the pathway are organized in five operons, which are inducible by the presence of the substrate, with some genes showing high basal expression levels. Two regulatory genes (redR1 and redR2) belonging to the NtrC family and showing 97% identity with each other are located in this region. The promoters of three operons coding for the three first steps of the pathway show the typical structure of sigma-54 dependent promoters (Prhl, Pbqdhl, and Porf14). Analysis of a series of knockout mutants showed that RedR2 regulator was essential for the expression of the three main promoters, and that resorcinol was not the direct effector of RedR2, but it needed to be metabolized to hidroxyhydroquinone (HHQ) to activate transcription. The sensor domain or the protein has been purified to confirm direct binding or HHQ to the regulator. In the absence of the second step of the pathway (btdhs) expression from the three promoters appears to be independent of the presence of the substrate. Double mutants have been constructed to identify which regulator is responsible for this effector-independent expression. *In vivo* assays of a truncated version of the regulatory proteins lacking the N-terminal sensor domain with promoter ::lacZ fusions in a heterologous host showed that RedR1 also participates in the expression or Porf14 and Pbqdhl promoters in a manner that was dependent on the presence of sigma-54 factor and IHF. Pormoter::lacZ fusions in broad-host range vectors have been constructed to confirm these results in Azoarcus strains. Serial deletions of the promoter upstream sequence allowed definition of .RedR1 binding site in Porf14 and Pbqdhl promoter sequence. To confirm these sites, different truncates forms or the protein, all of them including the DNA-biding region, have been purified and used in EMSA and foot-printing assays.

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P21-48
Dimerization through the conserved motif B is involved in chromatin association and function of bromodomain BET proteins

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BET (Bromodomain and Extra Terminal domain) proteins are unique among bromodomain-containing proteins in that they not only associate to acetylated chromatin in interphase but also remain attached to chromosomes during mitosis. Although the two tandem bromodomains are essential to display this behavior they do not account for full binding capacity. In fact, a deletion construct of the family member Brd2 encompassing just the two bromodomains fails to remain associated to chromosomes in metaphase. By deletion analysis, we have found that a small conserved domain, motif B, is required for that. We also show that motif B-dependent association to chromosomes is not restricted to mitosis, but extends to interphase. Interestingly, our results indicate that the motif B constitutes a coiled-coil surface for homo- and hetero-dimerization between BET proteins. This family of proteins play essential roles in cell cycle progression. Accordingly, we found that dimerization through the motif B is important for Brd2-mediated transactivation of cell cycle genes. Finally, linked to this prominent role in cell proliferation, we observed that ectopic expression of Brd2 interferes with neuronal differentiation in P19 cells and in the vertebrate neural tube, probably due to the maintenance of high levels of cyclins A2 and D1. By contrast, a deletion mutant of the motif B fails to perform in this way, highlighting the relevance of this domain for Brd2 function.

P21r-49
The prefoldin complex plays a role in transcription elongation

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RNA polymerase II requires the assistance of numerous general transcription factors during the elongation phase. In order to identify new molecular elements involved in transcription elongation, we performed a systematic genetic screening in *Saccharomyces cerevisiae* using the GLAM assay, a quick and easy method for detecting mutations affecting gene expression in a gene length-dependent manner [1]. We found a new factor not previously related to transcription elongation, the prefoldin complex. So far, prefoldin has been described as a heterohexameric cytoplasmatic complex, conserved from archaea to higher eukaryots, and involved in actin and tubulin biogenesis.

We analyzed the possible role of the yeast prefoldin complex in transcription elongation. We show that this complex is also localized in the nucleus, and that it is recruited to coding regions in a transcription-dependent manner. Additionally, we report that the *pfd1* mutant, lacking one of the subunits of the complex, shows transcriptional defects and an abnormal distribution pattern of RNA pol II along genes. Moreover, in the absence of Pfd1, TFIIS function in transcription elongation becomes much more important. The possible specific mechanism of action of the prefoldin complex during transcription elongation is discussed.

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P21-50
Dam methylase accessibility as an instrument for analysis of mammalian chromatin structure

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The study of regulatory mechanisms operating at the level of the chromatin fiber is one of the rapidly developing fields of modern molecular biology. Changes in chromatin condensation state is one of the main regulatory mechanisms of cell functions, such as transcription, replication, DNA repair, and other processes. The lack of information on dynamic behavior of extended chromatin regions leads to a gap in our understanding of mechanisms of coordinated expression regulation of gene ensembles. Chromatin structure analysis is in many cases based on the accessibility of chromatin DNA to modifying agents. The main benefits are granted by *in vivo* detecting agents such as Dam methylase, for example.

For a 140 kb human genome locus, an analysis of the distribution of Dam methylase accessible sites, DNase I sensitive and resistant regions, and acetylated histone H3 molecules was performed and compared with transcriptional activity of the genes within the locus. It was demonstrated that promoter regions of all highly and moderately transcribed genes are highly accessible to methylation by Dam methylase. In contrast, promoters of non transcribed genes showed a very low extent of Dam methylation. Some highly Dam methylase accessible regions are present in the intergenic regions of the locus suggesting that the latter contain either unidentified non-coding transcripts or extended regulatory elements like locus control regions.

P21-51
TFIIS is required for the balanced expression of the genes encoding ribosomal components under transcriptional stress

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TFIIS allows backtracked RNA polymerase II to resume transcription by stimulating extruded mRNA cleavage at the catalytic centre of the enzyme. TFIIS is conserved in all eukaryotes and it is encoded by the gene *DST1*. In *Saccharomyces cerevisiae* TFIIS is not essential except in conditions of transcriptional stress due to NTP depletion by addition of drugs like 6-azauracile (6AU) or mycophenolic acid (MPA). In order to study the role of TFIIS in this condition our group carried out a screening for suppressors of *dst1*Δ cells' sensitivity to 6AU. Four of the five isolated suppressors were related to ribosomal components (RiBi and RP genes) transcriptional regulation, including the general regulator Sfp1. We confirmed that this sensitivity can also be suppressed by other mutants related to the transcriptional regulation of ribosome synthesis. By chromatin immunoprecipitation we determined that TFIIS is present in RNA pol II as well as in RNA pol I transcribed regions, and it is specially recruited to RP genes upon 6AU treatment. In a *dst1*Δ mutant, after 6AU treatment the level of active polymerases is

affected on RP genes (transcribed by RNA pol II) but not in RNA pol I transcribed genes. This causes an unbalance in the synthesis of transcripts from both polymerases that implies deleterious consequences for ribosome biogenesis and cellular homeostasis. Nevertheless, the absence of Sfp1 produced an alteration on the response to NTP depletion that allowed the transcription of RP genes even in the absence of TFIIS. We propose that TFIIS is needed for the balance expression of RNA pol II and RNA pol I transcribed genes in conditions of transcriptional stress caused by a depletion of the levels of NTPs after 6AU or MPA addition.

P21-52
Effects of fibroblast growth factor (FGF acidic) and epidermal growth factor (EGF) on procollagen-N proteinases; ADAMTS-2 and ADAMTS-3 in osteosarcoma model

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A Disintegrin and Metalloproteinase domain, with Thrombo-Spondin (ADAMTS) is a family of zinc-dependent proteases that have common features with A Disintegrin and Metalloproteinase (ADAM) and also MMP enzymes (Matrix Metalloproteinases). ADAMTS's have important roles in a variety of normal conditions such as development, homeostasis, fertility and pathological conditions, including arthritis and cancer.

ADAMTS-2 and ADAMTS-3 cleave type I, II, III and V collagen precursors into mature molecules. ADAMTS-2 has important functions both in regulating the formation and structure of skin and in the maturation of spermatogonia. It is correlate with the incidence of both Ehlers–Danlos syndrome type VII C and dermatosparaxis in cattle. ADAMTS-3 has been identified as a type II procollagen N-propeptidase, It's expression is much lower than ADAMTS-2 in skin but is about 5-fold that of ADAMTS-2 in cartilage.

In this study we examined the effect of Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF acidic) on the mRNA expression level of ADAMTS-2 and ADAMTS-3 in a osteosarcoma model, SaOs-2 cells. This effect has been invesitigated by quantitative RT-PCR (qRT-PCR) in diffferent time intervals namely 1, 2, 3, 6, 24, 48 and 72 hour. Acidic FGF upregulates ADAMTS-2 and ADAMTS-3 expression at mRNA levels up to four fold in 6 hours. EGF upregulates ADAMTS-2 and ADAMTS-3 up to two fold for 24 hours.

P21r-53
hSNF5 is involved in cytokinesis and endocytosis by DNM2 interaction

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The tumor suppressor protein hSNF5 is an essential component of the mammalian SWI/SNF chromatin remodeling complex. It has been reported that this protein has a masked nuclear export signal and is transiently exported to the cytoplasm upon cell infection with HIV-1. We show the existence of a permanent fraction of hSNF5 in the cytoplasm by biochemical fractionation and immunofluorescence of cultured cells. Moreover, we demonstrate that exists a constitutive transport of EGFP-hSNF5 from the nucleus to the cytoplasm by fluorescence loss in photobleaching (FLIP), indicating that hSNF5 shuttles between the nucleus and the cytoplasm. In order to elucidate hSNF5 cytoplasmic functions, we performed a yeast two-hybrid screening to identify putative hSNF5 interacting proteins. We identified Dynamin 2 (DNM2) as a putative hSNF5 interacting protein and verified this interaction by coimmunoprecipitation, immunofluorescence colocalization and pull down experiments. DNM2 is a GTPase implicated in several cellular events including cytokinesis and endocytosis. It has been shown that this protein localizes in midbodies and that Dnm2 KO cells have cytokinesis defect. We show that hSNF5 is also present in midbodies and that hSNF5 overexpression prevents cytokinesis. Moreover, we have detected the opposite effect when antibodies against hSNF5 are transfected in cells. On the other hand, it has been demonstrated that DNM2 depletion by small interfering RNA (siRNA) inhibits endocytosis. We have seen that stability of DNM2 protein decreases in hSNF5-depleted cells, strongly impairing endocytosis. Taken together, our results reveal a new role of hSNF5 in cytokinesis and endocytosis, probably by its interaction with DNM2.

P21-54
Cohesin's potential role in immunoglobulin class switching

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The immunoglobulin heavy chain (IgH) locus changes its chromosomal conformation to assure stage-specific assembly of Ig molecules through several stages of B-cell development. Mature B cells undergo class switch recombination (CSR) upon antigen stimulation to diversify antigen specificity into different effector functions by changing the constant region of the Ig molecule. A DNA-loop is formed between the donor and acceptor S regions and the intervening DNA is excised and both ends are repaired by a mechanism involving the non-homologous end-joining pathway. Non-coding germ-line transcription (GLT) occurs to initiate CSR and it requires long-range interactions between the common Eμ enhancer, I promoters and 3′ regulatory region (3′RR) of the IgH locus. How these long-range interactions and how S/S synapsis are facilitated and which factors are involved still remain to be elucidated. The cohesin complex is a major candidate to facilitate DNA-looping to promote germ-line transcription, and to support S/S pairing given the facts that it topologically links two dsDNA molecules. Cohesin may also restrict these processes to the proper chromosomal regions. We aim to identify these potential roles of cohesin. We focus on differential binding of cohesin to the IgH locus in steady-state and switch-activated primary murine B cells. We determined the binding profile of cohesin at the IgH locus by ChIP-Seq and started to analyze the patterns of localization and potential co-localization factors.

P21r-55
Abortive initiations of chromosome replication under thymine starvation correlate with cell death in *Escherichia coli*

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Thymineless death (TLD) and it has been associated with uracil incorporation to DNA, generation of complex DNA structures and double strand breaks (DSB), induction of SOS etc, however, the precise contribution as 'primary cause' remains unknown. We have recently proposed the initiation events aborted under thymine starvation as the key element of TLD (Mata & Guzmán 2011, *DNA Repair* 10: 94–101). To verify whether abortive initiation of chromosomal replication events occur under thymine starvation, two approaches were used: DNA synthesis labeling assays and two-dimensional (2D) agarose gel electrophoresis. In this work we show that thymine starvation accumulates a chromosomal initiation capacity (ChIC). It is well-known that TLD is prevented by rifampicin addition. According to our model we found that in the presence of sub-inhibitory rifampicin concentrations, thymine starvation decreases ChIC; alleviating TLD. Using 2D gels, chromosomes of thymine-starved cells in the presence or the absence of rifampicin were analyzed. After 2 hours of thymine starvation, DNA intermediates corresponding with stalled replication fork around *oriC* region were observed. None of these DNA structures were detected under thymine starvation in the presence of rifampicin, where new initiation events are prevented. The results presented here indicate that, under thymine starvation, initiations of chromosome replication (i) indeed occur and (ii) they correlate with TLD. These observations reveal the initiation events as critical targets to develop new therapies improving the lethal effect of the treatment mimicking thymine starvation.

P21-56
Role of cMyb in the transcriptional regulation of cytosolic alanine aminotransferase in starved and insulin-treated gilthead sea bream (*Sparus aurata*)

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Alanine aminotransferase (ALT) plays an important role by linking amino acid, carbohydrate and energy metabolism. In *S. aurata*, the cytosolic ALT (cALT) gene generates two isoforms by alternative splicing: cALT1 and cALT2. In fish liver, cALT2 expression is associated to enhanced gluconeogenesis while cALT1 is predominant during postprandial use of dietary nutrients. To study control of cALT expression we characterized the effect of the transcription factor cMyb on *S. aurata* cALT promoter.

A fragment of *S. aurata* cMyb cDNA was isolated using RT-PCR and oligonucleotides designed from a multiple alignment of cMyb cDNAs reported for different species. Transient transfection of SBL cells with deleted cALT reporter promoter constructs and a cMyb expression plasmid, EMSA and site-directed mutagenesis revealed that cMyb transactivates cALT promoter by binding to a site located at nucleotide position -52 to -35 relative to the transcriptional start. Quantitative real time RT-PCR assays showed that cMyb mRNA levels increased in the liver of starved fish, whereas treatment with insulin down-regulated cMyb expression.

In conclusion, since modulation of cMyb expression correlated that described for cALT2 in the liver of *S. aurata*, our findings point to an important contribution of cMyb in the transcriptional control of cALT2 expression.

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P21-57
Parent-of-origin dependent DNA methylation does not necessarily associates with imprinted expression of nearby genes

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Parent-of-origin differential DNA methylation has been associated with regulation of the preferential expression of paternal or maternal alleles of imprinted genes. Based on this association, recent studies have searched for parent-of-origin dependent differentially methylated regions in order to identify new imprinted genes in their vicinity. In a previous genome-wide analysis of mouse brain DNA methylation, we found a novel DMR in a CpG island located in the last intron of the *alpha 1 Actinin (Actn1)* gene. In this region, preferential methylation of the maternal allele was observed; however, there were no reports of imprinted expression of *Actn1*. Therefore, we have tested if differential methylation of this region is common to other tissues and species and affects the expression of *Actn1*. We have found that *Actn1* differential methylation occurs in diverse mouse tissues. Moreover, differential methylation is also present in other murine rodents (rat), but not in the orthologous human region. In contrast, we have found no indication of an imprinted effect on gene expression of *Actn1* in mice: expression is always biallelic regardless of sex, tissue type, developmental stage or isoform. Therefore, we have identified a novel parent-of-origin dependent differentially methylated region that has no apparent association with imprinted expression of the closest genes. Our findings sound a cautionary note to genome-wide searches on the use of differentially methylated regions for the identification of imprinted genes and suggest that parent-of-origin dependent differential methylation might be conserved for functions other that the control of imprinted expression.

P21-58
Effects of methylprednisolone on DNA methylation in HL-60 AML cells

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Aim: Methylprednisolone(MP) has been used with short course and high dose for treating newly diagnosed children with AML.

Methylprednisolone induces differentiation of myeloid leukemia cells to granulocytes and macrophages and induces apoptosis of myeloid leukemia cells. In this study, methylation and expression of p15, ER, CDX2, BCL-2 genes which are responsible for proliferation and cell cycle investigated before and after methylprednisolone treatment in HL-60 cell line which is demonstrated if methylprednisolone shows its effect with epigenetic pathway.

Material and Method: After treating MP to HL-60 cells, cytotoxity, apoptosis and differentiation analysis were done. Differentiation markers CD13,CD33,HLA-DR,CD11b antibodies were analysed with flow cytometry. DNA isolations were made through MP treated and untreated HL-60 cells. Methylation Spesific PCR (MSPCR) of p15,ER,CDX2,BCL-2 genes were done. Expression of Bcl-2 and p15 genes are detected with Real-Time PCR.

Results: As a result of MTT test, significant decrease in cytotoxity is demonstrated in 5×10^{-3}M ($p < 0.001$). And also effective dose for apoptosis and differentiation is established as 5×10^{-3}M. In flow cytometry analysis, CD13, CD33 antibody rates decreased while CD11b and HLA-DR rates increased. p15 and CDX2 genes are unmethylated MP treated and untreated HL-60 cells while ER, BCL-2 genes are methylated. No difference is determined between MP treated and untreated cells for expression of p15 gene. However Bcl-2 expression changes 0.1 fold in MP treated HL-60 cells. This means MP significantly decreases Bcl-2 expression.

Discussion: It's determined that MP doesn't change DNA methylation profile in HL-60 cell line. MP decreasean anti-apoptotic gene Bcl-2 expresssion. Then, it's suggested that MP can effect other epigenetic pathways such as histon methylation and miRNA expression. Following this study, the methylation profile analysis will be demonstrated in pediatric AML patient bone marrow samples after and before MP treatment. Other pro- and anti-apoptotic genes' expression will be revealed.

P21-59
The *Saccharomyces cerevisiae* Hot1p regulated gene *YHR087W* has a role in translation upon high glucose concentration stress

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While growing in natural environments yeasts can be affected by osmotic stress provoked by high glucose concentrations. The response to this adverse condition requires the HOG pathway and involves transcriptional and posttranscriptional mechanisms initiated by the phosphorylation of this protein, its translocation to the nucleus and activation of transcription factors. One of the genes induced to respond to this injury is *YHR087W*. It encodes for a protein structurally similar to the N-terminal region of human SBDS whose expression is also induced under other forms of stress and whose deletion determines growth defects at high glucose concentrations. In this work we show that *YHR087W* expression is regulated by several transcription factors depending on the particular stress condition, and Hot1p is particularly relevant for the induction at high glucose concentrations. In this situation, Hot1p, together to Sko1p, binds to *YHR087W* promoter in a Hog1p-dependent manner. Several evidences obtained indicate Yhr087wp's role in translation. Firstly, and according to TAP purification experiments, it interacts with proteins involved in translation initiation. Besides, its deletion mutant shows growth defects in the presence of translation inhibitors and displays a slower translation recovery after applying high glucose stress than the wild type strain. Analyses of the association of mRNAs to polysome fractions reveals a lower translation in the mutant strain of the mRNAs corresponding to genes *GPD1*, *HSP78* and *HSP104*, which could explain the lower levels of some of these proteins found in previous proteomic analyses and the growth defects of this strain.

P21r-60
Transcriptome analysis of cisplatin treatment in yeast and the effect of *ixr1* and *sky1ixr1* mutations

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Cisplatin (cis-diamminedichloroplatinum(II)) is a widely used anti-cancer drug that forms DNA adducts, mainly 1,2-d(GpG) intrastrand cross-links. A cisplatin lession in the DNA strand blocks transcription and replication and also induces apoptosis.

The yeast Ixr1p protein is a structure-specific recognition protein (SSRP) that recognizes and binds to the cisplatin-DNA adducts through their High mobility group (HMG) domains. It was suggested that Ixr1p might have a role in mediating the citotoxicity of cisplatin blocking the excision repair machinery (Brown *et al.*, 1993). Sky1p is a yeast S/R protein-specific kinase and the null *sky1* confers resistance to cisplatin. Previous work suggests a link between *SKY1* and *NPR2* (Nitrogen permease regulator 2), but the mechanism by which *sky1* confers resistance to cisplatin remains to be elucidated (Schenk *et al.*, 2003).

Recently, we have found that the *Saccharomyces cerevisiae* W303 *sky1ixr1* strain has an additive effect in the response to the drug (Rodríguez-Lombardero *et al.*, 2012). We present a transcriptome analysis of the strain W303 and their mutants *ixr1* and *sky1ixr1* derivatives with and without cisplatin treatment. Results will be discussed in reference to the nature of the mechanisms causing cisplatin sensitivity or resistance and the role of Ixr1p and Sky1p in this response.

P21-61
Lack of Tdp2 causes etoposide-induced hematologic toxicity and genome instability in mice

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Type II topoisomerases (Top2) relax DNA molecules by catalyzing the passage of duplex DNA through a transient double strand break (DSB). Topoisomerases action involves the formation of an intermediate called the cleavage complex, which is normally very transient, being rapidly followed by resealing of the break. However, in the presence topoisomerases poisons or aberrant DNA structures, the cleavage complex becomes stable, producing a DSB with the enzyme covalently linked to 5′-termini via a phosphotyrosyl bond. The recently identified human tyrosyl DNA phosphodiesterase 2 (TDP2) is the only reported enzyme with the capacity to cleave 5′-phosphotyrosyl bonds and thereby release trapped topoisomerase II from DNA breaks. The previous observed sensitivity of cells lacking TDP2 to anti-cancer Top2 poisons such as etoposide, and the accumulation of etoposide-induced DSBs in such cells demonstrated that TDP2 exerts a

major impact on the repair of Top2-associated DSBs induced by Top2 poisons.

The characterization of a novel *TDP2* knock-out mouse model has allowed us to demonstrate that this repair pathway also operates *in vivo*. Consistent with the cellular data, *Tdp2*$^{-/-}$ mice show etoposide-induced hypersensitivity. Etoposide causes a significantly more accused splenic and thymic atrophy in *Tdp2*$^{-/-}$ animals, associated with a reduction in splenocytes and thymocytes. Interestingly, *Tdp2*$^{-/-}$ mice also display increased etoposide-induced genome instability in the bone marrow. Hematopoietic toxicity and genome rearrangements that lead to the development of secondary hematological malignancies are dramatic secondary effects Top2 poison-mediated chemotherapy. Our results, therefore, have important implications in the treatment of cancer.

P21r-62
Histone 3 K4, K14 and K56 post-translational modifications are not affected in hpr1Δ

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DNA is found in the cell associated with histones in chromatin. The basic unit of chromatin is the nucleosome, a 146-bp DNA wrapped around a protein octamer formed by histones H3, H4, H2A and H2B. Post-translational modifications of histones regulate different cellular functions such as transcription, replication or DNA repair. During transcription, H3K4 is trimethylated and H3K14 is acetylated facilitating RNAPII elongation through a chromatin template. Similarly, during replication newly incorporated nucleosomes are acetylated at H3K56 to facilitate de novo chromatin assembly. This modification has also been connected to nucleosome deposition after DNA damage repair.

Mutations in yeast THO, a conserved multiprotein complex that functions in transcription and mRNA export, cause accumulation of R-loops, nucleic acid structures formed by an RNA:DNA hybrid and a displaced single-stranded DNA. R-loop accumulation is toxic for the cell as it hinders transcription elongation, replication and increases genome instability. Thus, yeast THO mutants show pleitropic phenotypes from cell cycle delay to transcription impairment and an increased level of DNA damage. Here we sought to analyze whether these phenotypes induce the accumulation of post-transcriptional modifications of H3 linked to transcription or replication. We will present data on the analysis of H3K4 trimethylation, H3K14 or K56 acetylation in hpr1 mutants, as a way to determine the role of chromatin structure and remodeling in mRNP biogenesis and R-loop formation.

P21r-63
Understanding the regulation of CtIP roles via its many interactors

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CtIP is a multifunctional protein involved in several processes through interaction with many different partners. Such functions/partners include, among others, transcription repression (due to interaction with the transcription repressor CtBP), checkpoint activation (via interaction with MRN complex, the oncogenes Retinoblastoma and Brca1, and the proliferation factor PCNA) and double strand break (DSB) repair by recombination (interaction with Brca1 and the DSB repair factor MRN complex). Some of these interactions seem to be constitutive (CtBP), while others are cell cycle regulated (Rb, Brca1 and MRN). Therefore, CtIP is a key component in the response to DNA DSBs and the preservation of genomic stability that integrate multiple cell-cycle dependent signals. Despite the fact that CtIP has been found interacting with many different partners, it is still unknown if there are additional interactors of CtIP that can regulate its celullar roles.

To address this question, we have created a double-tagged (GFP and FLAG) CtIP fusion. Such construct has been integrated in U2OS cells. This cell line can be arrested with double thymidine treatments in the G1/S transition. Upon release from double thymidine, we have isolated proteins from large amounts of cells in different cell cycle phases (G1, S and G2). From these protein samples, CtIP complexes have been immunoprecipitated using an anti-FLAG antibody. After elution with an excess of FLAG peptide, a second immunoprecipitation round using an anti-GFP antibody was performed to ensure maximum purity of the complexes obtained. With this approach, we have isolated new interactors of CtIP.

P21r-64
CtIP as a novel tumor suppressor and its relevance for initiation, prognosis and treatment of cancer

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DNA damage and repair are closely linked to cancer, not only during tumor initiation and progression, but also as a potent antitumoral therapeutical opportunity. From the many types of damaged DNA, double strand breaks (DSBs) are specially relevant in oncology. DSBs are repaired by two major mechanisms that compete for the same substrate. Both ends of the DSB can be simple re-joined with little or no processing, a mechanism known as non-homologous end-joining. On the other hand, DSBs can be processed and engaged in a more complex repair pathway called homologous recombination. This pathway uses the information present in a homologue sequence. The balance between these two pathways is exquisitely controlled. Alterations in the DSBs repair pathways facilitate tumor progression and are selected early on during cancer development. On the other hand, DSBs are the molecular base of radiotherapies and several chemotherapies. Little is known about how cells chose between these two repair pathways, or the relevance of such choice in cancer initiation, progression or treatment. A main player for this election is the protein known as CtIP, which has been loosely implicated with cancer. We are studying the role of CtIP as a tumor suppressor and its importance in tumor initiation, progression, prognosis and treatment using cancer samples and cancer cell models.

P21-65
Uncovering the network that controls double strand break repair pathway choice

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Double strand breaks (DSBs) repair is essential for normal development. While the complete inability to repair DSBs leads to embryonic lethality and cell death, mutations that hamper this repair cause genetically inherited syndromes, with or without cancer predisposition. The phenotypes associated with these syndromes are extremely varied, and can include growth and mental retardation, ataxia, skeletal abnormalities, immunodeficiency, premature aging, etc.

DSBs are repaired by two major mechanisms that compete for the same substrate. Both ends of the DSB can be simple re-joined

with little or no processing, a mechanism known as non-homologous end-joining. On the other hand, DSBs can be processed and engaged in a more complex repair pathway called homologous recombination. This pathway uses the information present in a homologue sequence. The balance between these two pathways is exquisitely controlled and its alteration leads to the appearance of chromosomal abnormalities and contribute to the diseases aforementioned. However, and despite its importance, the network controlling the choice between both is poorly understood.

Here, we present a novel system specifically designed to unravel how the choice between both DSBs repair pathways is made, and its relevance for cellular and organismal survival, disease and development.

P21-66
Regulation of transcription in response to heat stress

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All cells, whether unicellular or part of a multicellular organism, must coexist with a variety of environmental fluctuations that might be harmful or even lethal. One of the most frequent environmental variations is the increase of temperature that leads to several changes in cells known as the heat stress response which includes protein denaturalization, transient cell cycle arrest and variations in membrane fluidity and structure. Consequently, the ability to rapidly respond to temperature fluctuations is essential for cell viability. Cells exposed to environmental stress alter the transcriptional program, resulting in the immediate down-regulation of housekeeping genes and the increase of defense and/or adaptation genes. Defense response to heat stress is characterized by a rapid induction of genes that encode for Heat Shock Proteins (Hsp), which keep protein homeostasis, relieving the folding defects and preventing protein aggregation and cellular damage. In order to define the global cellular adaptation response to heat stress, we performed a systematic genetic screen that yielded 277 yeast genes required for growth at high temperature. We found that the Rpd3 histone deacetylase complex was enriched. Global gene expression analysis showed that Rpd3 partially regulated gene expression upon heat shock. The Hsf1 and Msn2/4 transcription factors are the main regulators of gene activation in response to heat stress. *RPD3*-deficient cells had impaired activation of Msn2/4-dependent genes, while activation of genes controlled by Hsf1 was deacetylase-independent. Rpd3 bound to heat stress-dependent promoters through the Msn2/4 transcription factors, allowing entry of RNA Pol II and activation of transcription upon stress. Finally, we found that the large, but not the small Rpd3 complex regulated cell adaptation in response to heat stress.

P21-67
Important, distinctive role of the RSC complex in chromatin structure remodelling at the yeast *PHO* promoters

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The massive transition of chromatin structure at the yeast *PHO5* promoter from a repressed to an active, open state was demonstrated to be a prerequisite for promoter activation. We have previously shown that out of 15 non-essential chromatin-remodelling ATPases examined, chromatin remodelling at the *PHO5* promoter involved the activities of SWI/SNF and Ino80, but no remodelling complex critically required for the *PHO5* promoter opening has been identified yet. The RSC complex is an essential chromatin-remodelling complex in yeast and has been shown to disassemble nucleosomes *in vitro*. As inactivation of the RSC ATPase subunit Sth1 is lethal, we have used a temperature sensitive *sth1*^{td} mutant to assess the possible effect of RSC inactivation on the *PHO5* chromatin remodelling. The rate of chromatin opening and the consequent activation of *PHO5* promoter upon physiological induction in phosphate-free medium were significantly delayed by Sth1 depletion and this effect was even more pronounced under weaker induction conditions. Chromatin remodelling and consequent activation of a Gal4-activated *PHO5* promoter variant, induced through GAL-signalling pathway, was similarly affected by RSC inactivation showing that the observed effect was independent of the induction conditions and transactivator involved. Simultaneous inactivation of SWI/SNF and RSC complexes completely prevented remodelling of the native *PHO5* promoter, showing a functional interplay of the two complexes in the remodelling process. Also, inactivation of the RSC complex in the *isw1 chd1* double mutant, which by itself showed a significant delay in the kinetics of *PHO5* opening, completely abolished remodelling. Altogether these results pointed out a crucial, distinctive role of the RSC complex for the remodelling process in the absence of dedicated remodellers like SWI/SNF, Isw1 and Chd1. Interestingly, inactivation of the RSC complex alone or simultaneously with either SWI/SNF or Isw1 and Chd1, had no appreciable effect on chromatin remodelling at the *PHO8* and *PHO84* promoters, which are coactivated with *PHO5*.

P21-68
Analysis of the light-regulated transcriptional network controlled by the Neurospora GATA type transcription factors WC1, WC2 and SUB1

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The GATA type transcription factors (TF) White Collar 1 (WC1) and White Collar 2 (WC2) form the heterodimeric circadian activator White Collar Complex (WCC). The WCC is a blue-light receptor that is required for all light induced transcription in Neurospora. Recently it has been shown that SUB1, which is also a GATA type TF plays a role on late light induction of subset of genes. Here we have investigated the role of WCC and SUB1 on light dependent gene expression by ChIPseq and RNAseq and found a requirement of both TFs for light-dependent expression of a subset of genes.

P21-69
Hypoxia-induced microRNA-382 increases angiogenesis by targeting PTEN in gastric cancer cells

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Hypoxia is an essential feature of the tumor microenvironment that stimulates angiogenesis. Recent studies have revealed important roles for microRNAs (miR) in regulating angiogenesis. But, little is known about the role played by miRs in angiogenesis in response to hypoxia. In this study, we characterized miR-382 upregulated by hypoxia and its functional relevance in hypoxia-

induced angiogenesis. We analyzed miRs induced by hypoxia in MKN1 human gastric cancer cell using microRNA microarray and selected miR-382 as an angiogenic miR (angiomir). Expression of miR-382 was upregulated in a time dependent manner under hypoxic conditions. Conditioned media (CM) from MKN1 cells transfected with a miR-382 inhibitor (antagomir-382) in hypoxia significantly decreased the vascular endothelial cell (EC) proliferation, migration, and tube formation activities. In contrast, CM from MKN1 cells overexpressing miR-382 in normoxia significantly increased the EC proliferation, migration and tube formation. These results indicate that miR-382 induced by hypoxia has a property to promote angiogenesis. We predicted phosphatase and tensin homolog (PTEN) as a target gene of miR-382 using four algorithmic programs (Target Scan, PicTar, miRanda and Sanger miRbase Target). Overexpression of miR-382 or antagomir-382 resulted in down- or up-regulation of PTEN under normoxia or hypoxia, indicating PTEN is a functional target gene of miR-382. Taken together, it suggests that miR-382 induced by hypoxia promotes angiogenesis and acts as oncogene by repressing PTEN.

P21m-70
Molecular cloning, expression, purification and characterization of wild type and mutant marmoset alpha class glutathione transferase

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Glutathione S-transferases (GSTs; EC 2.5.1.18) catalyze the nucleophilic attack of the sulphur atom of the glutathione on the electrophilic groups of a wide range of hydrophobic compounds. In addition to role in detoxification, GSTs may be involved in the intracellular storage and transport of a variety of other hydrophobic, non-substrate compounds including hormones, metabolites and drugs.

In this study, we clonned and carried out expression, purification and characterization of wild type and site directed mutated A-class Glutathione transferase from marmoset (*Callithrix jacchus*).

The full-length open reading frame of mrGSTA1 was amplified from the library as template. After purification of the amplified pET-21α(+)–mrGSTA1, this fragment was ligated into NdeI/HindIII-restricted pET-21α(+) expression vector using T4DNA ligase. Both GSTA1 were overexpressed in *E. coli*. Enzymes were purified from the lysate by affinity chromatography using S-hexylglutathione-Sepharose6B.

The specific activities of wild type and mutant mrGST A1-1 were determined with different substrates by spectrophotometric assays at 30°C. Specific activities of mrGST A1-1 were detected with different concentrations of 5-androstene-3,17-dione, 5-pregnene 3,20-dione, 1-chloro-2,4-dinitrobenzene, Phenethlylisothiocynate, Trans-2-Nonenal and Cumene hydroperoxide .

Steady-state kinetic parameters of the steroid isomerase activity of mrGST A1-1 were determined and found that the enzyme displays a highly lower Km value than human GST A3-3. The catalytic efficiency of both mrGST A1-1 for isomerization of Δ5-PD was found to be seven times lower than human GSTA3–3, while both enzymes displayed similar K_m values towards this steroid. Enzymatic activities were measured as human GSTA3–3: 37, mutant mrGSTA1-1: 5,12 and wild-Type mrGSTA1-1: 2.14 mmol/min/mg respectively.

Also specific activities (mmol/min/mg) of both mrGSTA1-1 were compared with the other enzymes from different sources.

Keywords: Alpha class GST, identification, enzyme activity, marmoset

P21-71
Regulation of the structure-specific endonuclease Mus81-Mms4 during the mitotic cell cycle

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Mus81-Eme1/Mms4 is an evolutionarily conserved heterodimeric endonuclease that plays an important role in the maintenance of genomic integrity in eukaryotic cells. This endonuclease cleaves branched DNA substrates *in vitro*, such as model replication forks, 3′-flaps, D-loops, Y- and X-shaped structures, all of which are potential targets *in vivo*. We analysed different aspects of the regulation of budding yeast Mus81-Mms4 and found that whereas Mus81, the catalytic subunit of this complex, does not present detectable changes throughout the mitotic cell cycle, Mms4, the non-catalytic subunit, undergoes cell cycle-dependent phosphorylation. Our studies indicated that the phosphorylation of Mms4 occurs only after bulk DNA synthesis and before chromosome segregation, and that this modification depends on Cdc28 (CDK) and Cdc5 (Polo-like kinase) activities. Moreover, nuclease activity assays performed with different Mus81-Mms4 substrates showed that Mms4 phosphorylation is absolutely necessary for the normal function of the Mus81-Mms4 complex. Consistently, a phosphorylation-defective *mms4* mutant showed highly reduced nuclease activity and increased the sensitivity of cells lacking the RecQ-helicase Sgs1 to various agents that cause DNA damage or replicative stress. The mode of regulation of Mus81-Mms4 restricts its activity to a short period of the cell cycle, thus preventing its function during chromosome replication and the negative consequences for genome stability derived from its nucleolytic action. Yet, the controlled Mus81-Mms4 activity provides a safeguard mechanism to resolve DNA intermediates that may remain after replication and require processing before mitosis.

P21-72
Altered gene expression patterns of *Thermoplasma volcanium* 20S proteasome-VAT complex proteins in stress response

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To maintain cellular functionality under stress, damaged or modified proteins have to be rapidly recognized and degraded. Proteolytic systems of the cell, specifically 20S proteasomes play a major role in this process. Multiple versions of proteasome subunits both in nature and magnitude allow eukaryotic cells to be adapted to a wide array of physiological roles including stress response. Whether proteasome and its regulatory VAT proteins in *archaea* play key roles to survive environmental stresses remains to be experimentally verified. This study aims at analysis of differential expression of 20S proteasome and VAT genes in a thermoacidophilic archaeon *Thermoplasma volcanium* as a response to external stresses. To this end we have analysed the time course expression of proteasome genes by qRT-PCR, Western Blotting and enzyme activity assays. Our results showed that mRNA levels of proteasome genes (up to seven-fold) and VAT genes (up to four-fold) were significantly increased during heat-shock at 65 and 70°C for 2 hours. These genes also were over expressed at suboptimal pH (pH 4.0) and by exposure to H_2O_2. The qRT-PCR results were consistent with that of Western-blot

analysis and activity assays. On the other hand, when chymotrypsin-like activity of *T. volcanium* was inhibited by chymostatine, cell growth was not affected under optimum growth conditions. Under heat-shock, the presence of inhibitor significantly impaired cell growth, but cultures not exposed to inhibitor could survive. All together these findings suggested a critical role for 20S proteasome-VAT complex in *T. volcanium* for adaptation to various stresses.

P21-73
Glucocorticoid receptor concentration affects action

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Glucocorticoid receptor (GR) levels vary between tissues and individuals and are altered by physiological and pharmacological effectors. However, the effects and implications of differences in GR concentration have not been fully elucidated. Using three statistically different GR concentrations in transiently transfected COS-1 cells, we demonstrated, using whole-cell saturation ligand-binding experiments, that high levels of wild type GR (wtGR), but not of dimerization deficient GR (GR^{dim}), exhibited positive cooperative ligand-binding with a concomitant increased ligand-binding affinity. Co-immunoprecipitation and fluorescent resonance energy transfer of differently tagged GRs showed that positive cooperative ligand-binding correlates with ligand independent dimerization of the GR. The downstream consequences of variation in GR concentration, positive cooperative ligand-binding and ligand-independent dimerization were investigated using promoter reporter constructs and an endogenous GC responsive gene, GILZ.

The extent of ligand-independent transactivation correlated with GR concentration in all systems, with the ability to dimerize in some systems, and with cooperative ligand-binding only with the endogenous gene. The transactivation potency of dexamethasone was significantly increased at GR concentrations that demonstrate positive cooperative ligand-binding in both the promoter reporter and endogenous gene studies. This left shift of the dose response curve was not observed with the GR^{dim} mutant indicating a requirement for GR dimerization. Positive cooperative ligand-binding and the concomitant increase in potency of the glucocorticoid (GC) response suggest an important mechanism of action through which the GR is primed to respond to subsaturating GC concentrations and displays a significant level of ligand independent activity in tissues with high GR concentrations.

P21-74
The essential response regulator RpaB from the cyanobacterium *Synechococcus elongatus* PCC 7942

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The NblS-RpaB signaling pathway, the most conserved two-component system in cyanobacteria, regulates photosynthesis and acclimatization to a variety of environmental conditions and is involved in negative regulation of high light induced genes. We recently showed that the response regulator RpaB is regulated by specific (de)phosphorylation from the histidine kinase NblS and that RpaB and its phosphorylatable residue Asp56 are both required for viability of *Synechococcus elongatus* PCC 7942. Furthermore, the phosphorylated form of RpaB is present in *Synechococcus elongatus* PCC 7942 cells growing under standard laboratory conditions and high light stress affected the ratio of phosphorylated to non-phosphorylated RpaB. This was the first *in vivo* demonstration, in a cyanobacterium, of changes in the ratio of phosphorylated to non-phosphorylated two-component response regulators in response to environmental conditions.

While mutants engineered to increase or decrease the intracellular RpaB levels have a minor impact on cell size, a genetically engineered RpaB-GFP (RpaB fused to green fluorescent protein) confers an anomalous cell appearance in the absence of native RpaB. Confocal Microscopy and *in vivo* analyses of several key *S. elongatus* strains will be discussed in the context of the role of the NblS-RpaB signaling pathway in cell homeostasis and cell compartmentalization.

P21-75
Transactivation of the mitochondrial alanine aminotransferase promoter by HNF4α in kidney of *Sparus aurata*

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Alanine aminotransferase (ALT) links carbohydrate and amino acid metabolism through catalyzing the reversible transamination between L-alanine and 2-oxoglutarate to form pyruvate and L-glutamate. In previous studies we showed the presence of three ALT isoforms in glithead sea bream (*Sparus aurata*): two cytosolic isoenzymes (cALT1 and cALT2) and a mitochondrial isoform (mALT).

To better understand control of mALT expression, we characterized the *S. aurata* mALT promoter and studied modulation of mALT expression in the fish kidney under different nutritional and hormonal conditions. 5′-Deletion analysis of mALT promoter in transiently transfected HEK293 cells, site-directed mutagenesis and electrophoretic mobility shift assays allowed us to identify HNF4α as a new factor involved in the transcriptional activation of mALT expression. Since HNF4α did not enhance the transcriptional activity of *S. aurata* cALT promoter, these findings suggest that HNF4α exerts a specific effect on the mALT gene. Quantitative RT-PCR assays showed that starvation and administration of streptozotocin (STZ) decreased HNF4α levels in the kidney of *S. aurata*, leading to the downregulation of mALT transcription.

From these results we conclude that HNF4α transactivates the mALT promoter and that the low levels of mALT found in the kidney of starved and STZ-treated fish result from decreased expression of HNF4α.

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P21-76
Discerning the role of FurA as the master regulator of iron homeostasis in *Anabaena* sp. PCC 7120

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In *Anabaena* sp. PCC 7120, the ferric uptake regulator FurA is a constitutive and essential protein whose expression is induced

under iron deprivation. In the present study we analyzed the impact of FurA overexpression and iron availability on the transcriptional modulation of a broad range of *Anabaena* iron uptake, transport, storage, and cellular iron utilization mechanisms, including enzymes involved in siderophore biosynthesis, TonB-dependent siderophore outer membrane transporters, siderophore periplasmic binding proteins, ABC inner membrane permeases, ferritin Dps family proteins, and enzymes involved in tetrapyrrole biosynthetic pathway. By combining reverse transcription-PCR analyses, electrophoretic mobility shift assays and DNase I footprinting experiments, we defined a variety of novel direct iron-dependent transcriptional targets of this metalloregulator, including genes encoding at least five enzymes involved in tetrapyrrole biosynthesis pathway. The results unravel the role of FurA as the master regulator of iron homeostasis in *Anabaena* sp. PCC 7120, providing new insights into the Fur regulons in cyanobacteria.

P21-77
Genome-wide transcriptional response of the thermoacidophilic archaeon, *Thermoplasma volcanium* to environmental stress

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The heat-shock response is observed in all three domains of life (*Eucarya*, *Bacteria*, and *Archaea*) which triggers a dramatic and complex program of altered gene expression. In *Archaea*, however, in comparison to bacteria and eukaryotes much less is known about the mechanisms underlying the stress response. In this study, transcriptome analyses of hydrogen peroxide (H_2O_2), temperature and pH induced stress in a thermoacidophilic archaeon *Thermoplasma volcanium* were conducted. The genome-wide transcription profiling of the *T. volcanium* cells exposed to these stressors was examined with Roche NimbleGen custom designed 4x72k expression array. The microarray covered all 1501 transcripts using eight probes per transcript. Oxidative, pH and temperature stress changed the expression of 23 genes (19 down-, 4 up-regulated), 16 genes (all down regulated), and 10 genes (all down regulated) ≥1.5-fold, respectively, with a probability threshold of 98%. Under three stress conditions more genes were being down regulated than up regulated as might be expected during a stress response. A molecular chaperone (hsp20-related small heat shock protein) and GrpE gene selected among those identified by microarray analysis were further analyzed by quantitative real time RT-PCR which demonstrated a consistent trend in expression pattern between the two technologies. Also, Western blot analysis corroborated the similar results at protein level.

P21-78
Pathways for the repair of 5′ blocked double-strand breaks

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DNA double-strand breaks (DSBs) are extremely cytotoxic, as a single of these DNA lesions can lead to cell death if left unrepaired. Furthermore, misrepair of DSBs can lead to important genome reorganizations and mutations, which is one important hallmark of tumorigenesis.

DSBs induced by endogenous (such as reactive oxygen species) and exogenous (such as ionizing radiation) agents display heterogeneous structure, the majority of them presenting aberrant DNA ends. Repair of these blocked DSBs requires processing by different cellular activities, both general and specific to particular types of DNA ends. Despite the physiological importance of blocked DSBs, their heterogeneity in structure has been an obstacle to study the mechanisms governing their repair.

We have developed a system that allows us, for the first time, to specifically generate a population of homogeneous blocked DSBs. We rely on combining DNA Topoisomerase II poisons, which generate DSBs with the enzyme covalently linked to 5′ ends, with models deficient in TDP2, the only enzyme capable of directly reverting these protein-DNA adducts. In this way, we have the capacity to specifically generate 5′ blocked DSBs, providing us with a powerful and novel tool to shed light on the factors and pathways required for the repair of these highly threatening lesions.

P21-79
BCL-2 expression and chromosomal abnormalities in follicular lymphomas

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Introduction: Follicular lymphoma is a slow growing form of B-cell non-Hodgkin lymphomas with characteristic pattern of morphology. The majority of these lymphomas overexpress BCL-2 protein as a result of translocation t(14;18).
Aim: Our aim is to correlate BCL-2 immunohistochemical expression with chromosomal abnormalities in follicular lymphomas.
Methods: The study was performed on 18 cases of follicular lymphoma. Fresh lymph node specimens from patients with clinical suspicions of lymphoma were used for paraffin embedding and cell culture for conventional cytogenetic analysis. The expression of BCL-2 was immunohistochemical determined on serial section.
Results: BCL-2 immunohistochemical expression was intense positive in 83% of cases and moderate positive in 17% of cases. The cytogenetic abnormalities detected on our cases were t(14;18) in 67%, monosomy 7 in 16.5% and 13q14 deletion in 16.5% of cases. Our study revealed a strong positive correlation ($r = 0.63$, $p = 0.02$) between the presence of t(14;18) and BCL-2 immunohistochemical expression. However, BCL-2 is also overexpressed in the absence of translocation.
Conclusions: Our data emphasize the correlation between BCL-2 overexpression and the presence of t(14;18), but also suggest that in follicular lymphomas exist another mechanism of BCL-2 upregulation.

P21-80
Role of BceF BY-kinase in host–pathogen interaction, biofilm formation and survival to stress in the opportunist pathogen *Burkholderia cepacia* IST408: transcriptomic and phenotypic approaches

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Bacterial tyrosine kinases (BY-kinases) were first described as controlling exopolysaccharide biosynthesis, but now they are known to have a role in several cellular pathways, by controlling protein activity and location. In this work we have used global transcriptomic analysis, phenotype characterization and host–pathogen interaction studies to determine the role of the BY-kinase BceF in the physiology and virulence of the opportunistic lung pathogens belonging to *Burkholderia cepacia* complex. The transcriptomic approach identified 630 genes that were differentially expressed in the *bceF::*TpR mutant compared to the parental *B. cepacia* IST408. The mutant presented decreased expression in genes involved in stress response, motility, cell adhesion and outer membrane composition and increased expression in genes related to intracellular signaling, type VI secretion and iron metabolism. Accordingly, to these results we observed the *bceF* mutant was more susceptible to heat shock stress and UV exposure, presented less swimming motility and has increased intracellular levels of c-di-GMP at 24 hour than the wild type strains. Also, while the parental strain forms mature biofilm structures with well differentiated 3D microcolonies, the mutant forms a few small cell aggregates, being unable to form biofilms. BceF is also required for full *B. cepacia* virulence, as confirmed by the decrease mortality of *Galleria mellonella* larvae and by mutant's inability to adhere and invade human lung epithelial cells and to disrupt the epithelium cell junctions, which enables bacteria to reach paracellularly the basolateral surface. These findings confirm that the BY-kinase BceF is involved in many cellular processes, including bacterial features that are crucial for *Burkholderia* virulence.

P21-81
The effects of TNF-α on ADAMTS-1 and VEGF gene expressions in HEP3B cells

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Angiogenesis is essential for tumor growth, progression and metastasis. Angiogenesis is controlled by several endogenous stimulators and inhibitors. Among these stimulators, the vascular endothelial growth factor (VEGF) owns a private value because of its importance and specificity in angiogenesis. An important inhibitor of the angiogenesis is the first described member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin) family, ADAMTS-1, and it is demonstrated that ADAMTS-1 binds and sequestrates VEGF and this results with the suppression of endothelial cell proliferation.

Several factors may change the expression levels of ADAMTS-1 and VEGF. This study aims to evaluate the effects of TNF-α on ADAMTS-1 and VEGF gene expressions in Hep3B cells in a dose and time dependent manner.

Hep3B cells were treated with different doses of TNF-α for different time intervals. Total cellular RNAs were prepared from all groups and subjected to RT-PCR using specific ADAMTS-1 and VEGF primers. GAPDH amplification was carried out as a positive control. Amplification products were analyzed on agarose gel electroforesis and densitometric analysis were carried out.

P21-82
Study of nuclear proteins sirtuin 2 and 6 of human fibroblasts in normal and accelerated ageing

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One of the ways human ageing mechanism could be explored is the study of progerias patients cells. Patients with progerias, or early ageing syndromes, have tissue and cell senility symptoms at the young age. Despite the fact these diseases were firstly studied in the XIX century, there is still no clear perception of the molecular framework and processes for these rare and serious diseases. To date, at the genetic level it is considered to have been proved the implication of protein products of genes, mutated in progeria, in the natural mechanisms of senescence. But despite the fact these molecules were characterized and their partners identified at epigenetic regulation level still remain questions. Among these proteins we name sirtuins and HP1γp.

Sirtuins are the class III histone deacetylases. It is known that expression level and functional status of sirtuins molecules change with increasing cell age. In this case, the nuclear Sirt6p and the cytoplasmic Sirt2p are especially interesting and important, including their behavior in young and old donors cells. These proteins have been shown to be directly or indirectly involved in some cell activity processes in connection with ageing. Our study aim is to determine the age dynamics of some sirtuins presence in human skin fibroblasts in normal and accelerated aging. Using Western blot or indirect fixed cells immunofluorescence staining methods there were shown certain differences of HP1γp presence in healthy donors and ataxia-telangiectasia (AT) patients cells (AT is a complex syndrome with some premature ageing symptoms, also called Louis-Bar syndrome). We have shown nuclear Sirt6p and cytoplasmatic Sir2p presence difference in skin fibroblasts of St. Petersburg patients with syndrome Louis-Bar (AT) comparing healthy donors cells in normal ageing process.

P21-83
DNA methylation as an additional diagnostic tool for tumoral mediastinal lymph nodes in non-small cell lung cancer

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Mediastinal lymph node metastasis in non-small cell lung cancer (NSCLC) is one of the main prognostic factors. Although transbronchial ultrasound-guided puncture (EBUS-TBNA) is minimally invasive for cytological analysis, false negatives are relatively high. Since epigenetic alterations regulate gene expression, we hypothesize that hypermethylation of the promoter

region of tumor suppressor genes *p16/INK4a*, *MGMT* and *SHOX2* could improve sensitivity and negative predictive value.

A total of 157 EBUS-TBNA samples from 77 patients with confirmed diagnosis of NSCLC were included (40.8% with ganglionar metastasis). According to cytology patients were considered True Positives (TP: +cytology/+metastasis), True Negatives (TN: -cytology/-metastasis) and False Negatives (FN: -cytology/+metastasis). DNA was extracted and modified with sodium bisulfite. Methylation levels were determined using a nested-MS-qPCR: initial PCR with universal outer primers followed by a qPCR with methylation-specific primers and probes targeting CpG islands. A standard curve (100–0.1% methylation) was constructed to quantify methylation, while DNA input was normalized by reference gene *MYOD1*.

The median methylation percentage of p16, *MGMT* and *SHOX2* for TP was 5.3%, 5.1% and 34.2%; for TN 5.2%, 5.1% and 6.1%, and for FN 5.2%, 5.1% and 6.7%. Clinical application was assessed through ROC curves, showing the same AUC of 0.56 (0.47–0.65) for p16 and *MGMT*; for *SHOX2* this was 0.85 (0.78–0.91). Methylation analysis of SHOX2 shows promising performance considering the 3 groups of patients ($p < 0.001$). Although no statistically significant differences were found between TN and FN ($p = 0.19$), the inclusion of a larger number of FN patients ($n = 12$) is mandatory to reveal the additional diagnostic potential of SHOX2.

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P21-84
Putative enhancer modules are harbored in Intron5 of the RUNX1 gene

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Human RUNX1 gene is the most frequent target for chromosomal translocations associated with acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). The highest prevalence in AML is noted with (8;21) translocation; which represent s 12–15% of all AML cases. Interestingly, all the breakpoints mapped to date in t(8;21) are clustered in intron 5 of the RUNX1 gene and intron 1 of the ETO gene. No homologous sequences have been found at the recombination regions; but DNaseI hypersensitive sites (DHS) have been mapped to the areas of the genes involved in t(8;21). Presence of DHS sites is commonly associated with regulatory elements such as promoters, enhancers, silencers, etc. In this study we used a combination of comparative genomics, chromatin analysis and transfection studies to evaluate potential regulatory elements located in intron 5 of the RUNX1 gene. Our genomic analysis identified nine potential enhancer regions that are evolutionarily conserved. Analysis of histone modification status of these regions in myeloid cells shows a high association of marks characteristic of enhancer modules. Two of these regions were cloned in front of a reporter gene and their effect on transcriptional regulation was evaluated. Taken together our results identified two conserved non coding sequence in intron 5 of the RUNX1 gene that can regulate transcription expression of a reporter gene.

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P21-85
The ING1a tumour suppressor regulates endocytosis to induce cellular senescence via the Rb-E2F pathway

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Background: The INhibitor of Growth (ING) family of genes are a class of type II tumour suppressors, that specifically recognize and bind to the histone epigenetic mark HeK4Me3 in a methylation-sensitive manner and affect gene expression. INGs also target HAT and HDAC complexes to chromatin, to affect local histone acetylation levels. ING1 encodes two major splicing variants-ING1a and ING1b. As cells approach replicative senescence, the ING1a:ING1b ratio in cells, increases by >30 folds. ING1a, the longest isoform of ING1, induces many features of replicative senescence in primary fibroblasts including growth inhibition, a large flattened morphology, expression of SAβG, increased p16 and cyclin D1, and accumulation of SAHF. In contrast, ING1 knock-down extends the replicative lifespan of cells, but by an unknown mechanism.
Key findings: Here, using an unbiased global screen, we find that ING1a over-expression transcriptionally affects a number of endocytic genes including intersectin2 (ITSN2), the Janus kinase and EPS15. ITSN2, the endocytic adaptor protein, is the highly upregulated gene upon ING1a over-expression. ITSN2 levels are higher in senescing cells compared to young fibroblasts and knocking down ING1a in senescent cells affects the expression of ITSN2. Furthermore, we find that ING1a binds to the ITSN2 promoter and affects receptor internalization, thus delaying endocytosis. Interestingly, we also find that inhibition of endocytosis by several chemical and biological approaches in young replication-competent cells result in all of the senescent-like phenotypes observed in ING1a expressing cells, confirming that regulation of endocytosis plays a major role in ING1a induced senescence. Furthermore, we also found an attenuation of most of the major signaling pathways in ING1a expressing cells, leading to inhibition of E2F target genes, by keeping them bound to the Rb tumour suppressor.
Significance: These data indicate that the senescence associated alternative splicing of ING1a contributes to inducing replicative senescence and provides mechanistic insight into how dysfunctional endocytosis of growth factor receptors plays a direct role in inducing the senescence phenotype. Results emphasise a novel role of epigenetic regulation of growth factor signalling in cellular senescence.

P21-86
Jmjd3 is a novel target of Stat1 and Stat3 which cooperate to drive inflammatory genes expression in LPS-stimulated microglia

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Uncontrolled and prolonged inflammation is associated with earlier onset and/or progression of virtually all neurological diseases including Alzheimer's and Parkinson's disease, brain trauma and stroke. Microglial cells accumulate in regions of degeneration and produce a wide variety of pro-inflammatory molecules. The

Janus Kinase (JAK)-Signal transducer and activator of transcription (STAT) pathway converts the cytokine or TLR signals into gene expression programs that regulates immune and glial cell functions. Stat targets and molecular mechanisms underlying inflammatory activation of microglia are unknown. In primary microglial cultures lipopolysacharide (LPS) induced stimulation leads to rapid activation of Stat1, 3, and 5. We mapped the genome-wide occupancy of active, phospho-Stats by hybridization of immunoprecipitation-enriched genomic DNA to promoter microarrays (3x720K RefSeq Promoter microarrays, NimbleGen). We found correlation of active Stat1 and three binding sites with changes in expression of many genes encoding cytokines/chemokines and transcription regulators. The most interesting hit, representing a newly identified Stat target, was *jmjd3*, encoding a JmjC family histone demethylase and transcription factor, which controls inflammatory gene expression in peripheral macrophages. Silencing of Stat1 and Stat3 blocked Jmjd3 and inflammatory gene expression in Bv2 microglial cells, while overexpression of constitutively active Stat1 and Stat3 was sufficient to induce Jmjd3 and inflammation-related genes in the absence of LPS. Action of Jmjd3 did not depend on its histone demethylase activity, but was lost after interference with its transactivator domain. These data show that Stat1 and Stat3 are necessary and sufficient for initiation an appropriate inflammatory response.

P21-87
TDP2 functions during non-homologous end-joining and is required for genomic stability in response to topoisomerase II poisons

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Type II topoisomerases (Top2) relax DNA molecules by catalyzing the passage of duplex DNA through a transient double strand break (DSB). DNA cleavage by topoisomerases involves the formation of an intermediate called the cleavage complex, which is normally very transient, being rapidly followed by resealing of the break. However, in the presence specific drugs (known as topoisomerases poisons) or aberrant DNA structures, the cleavage complex becomes stable, producing a DSB with the enzyme covalently linked to 5′-termini via a phosphotyrosyl bond. The recently identified human tyrosyl DNA phosphodiesterase 2 (TDP2) is the only reported enzyme with the capacity to cleave 5′-phosphotyrosyl bonds and thereby release trapped topoisomerase II from DNA breaks.

We show that TDP2 facilitates non-homologous end joining (NHEJ) mediated repair of 5′-phosphotyrosyl-blocked DSBs *in vitro*. Furthermore, using *TDP2* knock out cellular models, we confirm the relevance of this enzyme to tolerate and repair Top2-induced DSBs. Finally, the absence of TDP2 causes an increase in homologous recombination and genome instability in the presence of Top2 poisons that are widely used in cancer chemotherapy. Overall, our results are consistent with TDP2 acting in a novel error-free subpathway of NHEJ, which is relevant to avoid genome instability upon treatment with Top2-poisons.

P21-88
Epigenetic studies revealed a new sesquiterpene family involved in conidiation in *Botrytis cinerea*

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The gray mold *Botrytis cinerea* Pers. is a necrotrophic plant pathogen causing serious pre- and postharvest losses in more than 230 crop species worldwide. Despite the introduction of new antifungal compounds, *B. cinerea* is difficult to control because of its broad host range, high genetic variation, and ability to survive as a saprophyte for extended periods as sclerotia in crop debris. Dispersal of *B. cinerea* is predominantly via conidia and this impact in the epidemiology and the control methods employed.

The wide variety of symptoms on different organs and plants indicates that *B. cinerea* has a large arsenal of chemical weapons to attack its host plants. *Botrytis* produces two series of phytotoxic metabolites: a family of characteristic sesquiterpene metabolites which contain the basic botryane skeleton and two polyketide lactones types: botcinins and botrylactone.

The sequencing of the genomes of the B05-10 and T4 strains of the fungus *B. cinerea* revealed an abundance of novel biosynthetic gene clusters, the majority of which were unexpected on the basis of previous fermentation analyses of these and closely related species. This data revealed that *B. cinerea* has 43 key enzymes involved in the biosynthesis of secondary metabolites, including many polyketides, peptides and terpenes, some of them are specific of this phytopathogen. The study displayed six genes coding for putative sesquiterpene cyclases (STC) in *B. cinerea*.

Using chemical epigenetic fermentation methodology we have found a cryptic sesquiterpenic family with a structure related to eremophil-9-en-1-ol. Determination of absolute stereochemistry by spectroscopic methods indicated that this new group of compounds has an enantiomeric structure to that of the sesquiterpene 5-epi-aristolochene. *In vitro* evaluation of the biological role of these metabolites indicates that they contribute to the conidial development in *B. cinerea*.

P21-89
The transcription factor FL in the fungus *Neurospora crassa* is phosphorylated and interacts with a trehalose related protein

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Several environmental cues, including blue light, promote a developmental transition in the fungus *Neurospora crassa* that leads to the development of spores (conidia). Conidiation is controlled by FLUFFY (FL), a transcription factor with a binuclear zinc cluster domain. Blue light activates the transcription of *fl* through the transient binding of the WC complex to the *fl* promoter. The WC complex is a light-dependent transcription factor complex that is required for all the responses of *Neurospora* to light. Light activates the transcription of several conidiation genes in the fungus *Aspergillus nidulans*, and their *Neurospora* homologs have been identified in the *Neurospora* genome. We have assayed the activation by light of the *Neurospora* homologs of *A. nidulans* conidiation genes (*flbA*, *flbC*, *flbD*, *stuA and medA*), and the *Neurospora* conidiation gene *con-10* as a control. Unlike *con-*

10, none of the *Neurospora* homologs of the *A. nidulans* conidiation genes were induced by light in vegetative mycelia. However, we found that deletion of the regulatory gene *fl* resulted in light-dependent mRNA accumulation for all the conidiation genes. This result indicated that the absence of FL allows the binding of the WC complex to the promoter of these genes to activate transcription in a light-dependent manner. We have assayed the amount of WC proteins in the *fl* and wild type strains but we did not find any difference between the two strains suggesting that FL and the WCC may compete for binding sites in close proximity in the promoter of these genes. We expect to identify additional genes deregulated by the absence of FL after massive sequencing of total RNA experiments (RNAseq) using a *fl* mutant and wild-type strain in dark and light conditions. We have investigated the role of FL during conidiation in *Neurospora* using a tagged version of FL (FL::3XFLAG). We have found that FL is present in vegetative mycelia but the amount increses after blue-light exposure. In addition we have found similar amounts of FL during the vegetative and conidiation stages. In experiments with protein synthesis inhibitors we have found that FL is a very stable protein. Our results show that the regulatory role of FL cannot be exherted through the synthesis of the protein. We have observed several forms of FL due to phosphorylation, and and we have determined by mass spectrometry that FL::3XFLAG is phosphorylated in several residues. We have immunoprecipitated FL::3XFLAG in order to identify by mass spectrometry possible proteins interacting with FL. We have found that an hypothetical protein related with the ability to grow in the presence of trehalose in other organisms interacts with FL. The interaction has been tested in several conditions and seems to be very stable. Since FL is a transcription factor, we have use FL::3XFLAG strain to do ChIPseq (Chromatin Immunoprecipitation Sequencing) in order to identify the putative binding sites of FL to the DNA. We expect that the results from these experiments will help us to understand in more detail the role of FL in the activation of gene transcription during asexual development in *Neurospora crassa*.

P21-90
DAXX-dependent recruitment of histone variant H3.3 to PML bodies before deposition into chromatin

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Histone variant H3.3, in contrast to canonical H3.1 and H3.2, is synthesized throughout the cell cycle and incorporated into chromatin in a replication-independent manner (i.e., both during and outside S phase). Despite the recent identification of several H3.3 chaperones, the pathways of deposition of H3.3 into chromatin remain unclear. We determined the path of newly synthesized epitope-tagged H3.3 transiently expressed in human primary cells. Early after transfection, and prior to deposition into chromatin, H3.3 is targeted to PML bodies where it co-localizes with the H3.3-specific chaperones ATRX, DAXX, HIRA and ASF1A. Co-expression of histone H4 facilitates recruitment of H3.3 to PML bodies and accelerates its deposition into chromatin, suggesting that H3.3 is targeted to PML bodies and chromatin association with H4. An H3.3[H113A] mutant unable to from a stable $(H3.3[H113A]\text{-}H4)_2$tetramer in silico shows faster recruitment to PML bodies relative to wild type H3.3, suggesting that (H3.3-H4) is preferentially recruited as a dimer (rather than as a tetramer) to PML bodies. Fluorescence recovery after photobleaching (FRAP) analyses indicate that H3.3 recruitment to PML is DAXX-dependent. DAXX also affects the localization of ATRX but not ASF1A or HIRA at PML bodies, suggesting that DAXX and ATRX form a complex distinct from ASF1A and HIRA at PML. Down-regulation of ATRX does not affect DAXX or H3.3 targeting to PML. ASF1A facilitates DAXX-mediated H3.3 recruitment to PML bodies, but is not essential. Our data support a model of DAXX-mediated targeting of (H3.3-H4) dimers to PML bodies to facilitate H3.3 association with different chaperones, and subsequent site-specific incorporation into chromatin.

P21-91
Manganese homeostasis, genome stability and cell cycle

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Manganese acts as a cofactor for many enzymes and alterations in manganese homeostasis have been shown to affect metabolic functions. For example, mutations in the human Mn2+/Ca2+ transporter ATP2C1 have been linked to Hailey-Hailey disease formation. By deletion of the yeast orthologue *PMR1* we found that impaired manganese homeostasis contributes to genome instability and impairs proper cell cycle progression. Surprisingly, addition of extracellular calcium suppresses many phenotypes associated with the lack of Pmr1. New data will be presented on possible mechanisms that contribute to the calcium dependent alleviation of *pmr1*Δ caused phenotypes.

P21-92
Control of anaphase by the S phase checkpoint in budding yeast

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In eukaryotic cells a surveillance mechanism, the S phase checkpoint, responds to genotoxic insults to preserve genomic integrity. One branch of the S phase checkpoint, the S-M pathway, delays progression into mitosis to provide time to overcome the stress, thus preventing aberrant chromosome segregation and inheritance. How such block is achieved has long been well characterized in the fission yeast *Schizosaccharomyces pombe*. The paralog kinases wee1 and mik1 phosphorylate the Thr14 and Tyr15 residues of the Cyclin Dependent Kinase (CDK) catalytic subunit cdc2, which results in the inhibition of mitotic CDK activity (M-CDK). Puzzingly, both the ortholog Swe1 kinase and phosphorylation of the conserved Thr18 and Tyr19 residues were soon found to be dispensable in the budding yeast *Saccharomyces cerevisiae*. Such observations led to the widely assumed conclusion that such control is not conserved in budding yeast, where the checkpoint would essentially block anaphase through the stabilization of Pds1/securin. However, our results show that mutant cells that lack securin remain fully competent to block anaphase in response to genotoxic stress in S phase. We will provide results showing that Swe1 is indeed involved the checkpoint regulation of M-CDK, together with a parallel, Rad53 dependent pathway. Such redundancy explains the dispensability of Swe1 in budding yeast, and supports a universally conserved role of wee1/Swe1 kinases in the response to genotoxic stress. We have generated mutant strains that bypass the S phase checkpoint regulation of M-CDK activity. When combined with mutations that bypass the checkpoint ability to keep Esp1/separase activity inhibited, cells attempt the segregation of incompletely replicated chromosomes.

P22 – RNA Biology

P22-1
Interactions between cypovirus infection and the RNAi machinery in silkmoth and silkmoth-derived Bm5 cells

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Cytoplasmic polyhedrosis viruses (CPVs) are segmented dsRNA genome viruses that belong to the genus *Cypovirus* of the family *Reoviridae*. While the involvement of the RNAi machinery to control infections of ssRNA genome viruses is well established, nothing is known regarding the interaction between cypovirus infection and the RNAi machinery in insects.

In this study, effects of knockdown of the RNAi machinery on *Bombyx mori* CPV production are investigated. After BmCPV genomic dsRNA is co-transfected into silkmoth-derived Bm5 cells together with dsRNAs targeting the core RNAi machinery (Dcr2, Ago1, Ago2, Ago3), viral genome amplification is monitored during time by PCR and the production of infectious viral particles is determined.

Another series of experiments addresses whether BmCPV infection affects expression of genes of the RNAi machinery. In parallel, relevant ORFs of BmCPV (non-structural protein 5, with putative dsRNA-binding activity; and RNA-dependent RNA polymerase) have been cloned into expression vectors to check whether their expression in Bm5 cells results in inhibition of dsRNA-mediated gene silencing.

These studies attempt to clarify the natural function of the RNAi pathway in the silkmoth with the ultimate goal of developing more effective approaches to RNAi-mediated inhibition of gene expression in lepidopteran insects.

P22-2
Folding of the hepatitis C virus internal ribosome entry site is fine-tuned by the 3′ end of the viral genome

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The hepatitis C virus (HCV) genome is an RNA molecule containing a single open reading frame flanked by highly structured and conserved untranslatable regions (UTRs), with essential roles in the viral cycle. Additional functionally and structurally conserved RNA domains have been described, which are mainly associated with the UTRs. All these regions work together for the consecution of protein synthesis, replication and infectivity in *cis*. During early viral infection, uncapped viral RNAs are translated via an internal ribosome entry site (IRES) located at the 5′UTR. It is a highly structured motif that acts as scaffold for recruiting multiple protein factors. The translation initiation process is also guided by long-distant regions in the 3′ end of the HCV genome. We have applied classical RNA chemical probing methods, SHAPE (selective 2′-hydroxyl acylation and primer extension) structural analysis and the screening of RNA accessibility by antisense oligonucleotide microarrays to demonstrate that IRES folding is highly dependent on the 3′ end of the viral RNA. Our results show that IRES subdomains IIIb, IIId and domain IV adopted a significant differential conformation with respect to that exhibited by these elements in the absence of the 3′ end of the HCV genome. The observed effects occur in the lack of protein factors. This suggests the existence of a complex network of direct and long-distant RNA–RNA interactions involving essential domains for the viral cycle. These interactions could play important roles in the regulation of viral translation and replication, as well as in the switch between different steps of the HCV cycle.

P22-3
Characterization of the pre-60S ribosomal particles able to translate in Saccharomyces cerevisiae

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Ribosomes are large ribonucleoprotein particles that are composed of two subunits (40S and 60S in eukaryotes). Ribosomes assemble in a highly dynamic process that involves 79 ribosomal proteins, four rRNAs, more than 200 protein factors and about 100 snoRNAs. All these factors, whose precise functions are still largely unknown, likely allow the ribosome maturation process to proceed with the required speed, accuracy and directionality. Ribosome biogenesis starts in the nucleolus, continues in the nucleoplasm and is completed after nucleo-cytoplasmic export of the subunits in the cytoplasm.

In wild type conditions, pre-rRNA processing is coordinated with the nucleo-cytoplasmic export of pre-ribosomal particles. During 60S maturation, 7S pre-rRNAs are 3′ processed to 6S by the nuclear exosome; 6S pre-rRNAs are processed to mature 5.8S rRNAs in the cytoplasm. Only those pre-60S particles containing 6S pre-rRNAs are actively exported to cytoplasm. In any case, immature pre-60S particles are translating inactive.

Our group is interested in understanding how yeast 60S ribosomal subunits achieve translation activity. We have observed that in some mutant backgrounds, the pre-60S particles containing 7S pre-rRNAs are actively exported to the cytoplasm. More interesting, these immature pre-60S particles fractionate in the polysome region of sucrose gradients, hence, strongly suggesting that they are able to translate. Experiments are in progress to define the features that allow these pre-60S particles to escape the nuclear retention mechanism and engage into translation.

P22-4
Circadian amplitude of mouse *Cryptochrome 1* is regulated by cytoplasmic microRNA-185 oscillation

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Mammalian circadian rhythm is observed not only at the suprachiasmatic nucleus, a master pacemaker, but also throughout the peripheral tissues. Until now, research into the expression of

clock genes has mainly focused on transcription and post-translational modification, and little is known about the post-transcriptional regulation of these genes. In the present study, we investigated the role of the 3′-untranslated region (UTR) of the mouse *Cryptochrome 1* (m*Cry1*) gene at the post-transcriptional level, particularly in regards to microRNA (miRNA) mediated regulation. Knockdown of *Drosha*, *Dicer*, or *Argonaute2* resulted in increased levels of the m*Cry1*-3′UTR reporter, while the presence of the miRNA recognition element (MRE) of m*Cry1*, which is important for miR-185 binding, led to a decrease in protein level but not mRNA quantity. Indeed, mutation of the miR-185 binding region resulted in increased reporter levels, while the overexpression of miR-185 led to decreased reporter activity. Interestingly, cytoplasmic miR-185 levels were found to be nearly anti-phase to mCRY1 protein levels, and miR-185 knockdown elevated the amplitude of mCRY1 protein oscillation. Our results suggest that miR-185 plays a role as a fine regulator that contributes to the m*Cry1* mRNA translation rate of the rhythmicity of mCRY1 protein expression.

P22-5
Functional analysis of the role of ICln in snRNP biogenesis and splicing in *S. pombe*

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Numerous studies in mammals indicate that assembly of the eukaryotic spliceosomal snRNPs is a multistep process following an ordered pathway. The methylosome and the SMN (Survival of Motor Neuron) complexes are essential players in early steps of this pathway. The methylosome, composed by ICln, WD45 and PRMT5 proteins, recruits Sm proteins via the ICln subunit and symmetrically dimethylates arginines within the C-tails of Sm proteins. The SMN complex further facilitates the formation of the Sm core heptameric ring and the loading of this complex onto the snRNAs resulting in the formation of snRNP particles. Previous *in vitro* studies showed that ICln is an important regulator of snRNP assembly since it acts as an assembly chaperone while the SMN complex acts as a catalyst. In order to study the function of ICln *in vivo*, we used the *S. pombe* model organism which is a good model to analyze snRNP biogenesis and splicing. By sequence comparison, we identified an uncharacterized ICLn homologue in *S. pombe*. While deletion of the SpICln gene is not lethal, the icln strain shows a slow growth phenotype indicating an important role of SpICln for optimal yeast cell growth. The SpICln protein associates with the full set of Sm proteins and the human ICln gene complements the slow growth phenotype demonstrating that the identified SpICln sequence represents the bona fide human homolog. We found also that the SpICln protein is required for optimal stability of the spliceosomal snRNAs and for efficient splicing. Using a genome-wide approach and RT-PCR validation tests, we demonstrate that splicing is altered differentially since a subset of introns are preferentially retained in the icln cells. Statistical analyses indicate that the size and position of the PPT located upstream the branchpoint adenosine represent an important determinant which could explain the observed splicing inhibition in the group of retained introns. We are currently validating this model by constructing reporter genes carrying introns with longer polypyrimidine tracts and which should be spliced more efficiently in icln cells.

P22-6
Species-specific microRNA roles elucidated following astrocyte activation

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MicroRNAs (miRNAs) are short non-coding RNAs that play a central role in regulation of gene expression by binding to target genes. Many miRNAs were associated with the function of the central nervous system (CNS) in health and disease. Astrocytes are the CNS most abundant glia cells, providing support by maintaining homeostasis and by regulating neuronal signaling, survival and synaptic plasticity. Astrocytes play a key role in repair of brain insults, as part of local immune reactivity triggered by inflammatory or pathological conditions. Thus, astrocyte activation, or astrogliosis, is an important outcome of the innate immune response, which can be elicited by endotoxins such as lipopolysaccharide (LPS) and cytokines such as interferon-gamma (IFN-γ). The involvement of miRNAs in inflammation and stress led us to hypothesize that astrogliosis is mediated by miRNA function. In this study, we compared the miRNA regulatory layer expressed in primary cultured astrocyte derived from rodents (mice) and primates (marmosets) brains upon exposure to LPS and IFN-γ. We identified subsets of differentially expressed miRNAs some of which are shared with other immunological related systems while others, surprisingly, are mouse and rat specific. Of interest, these specific miRNAs regulate genes involved in the tumor necrosis factor-alpha (TNF-α) signaling pathway, indicating a miRNA-based species-specific regulation. Our data suggests that miRNA function is more significant in the mechanisms governing astrocyte activation in rodents compared to primates.

P22-7
The synthesis of novel HCV core + 1/ARF protein as a model system for studying unconventional translation mechanisms

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Hepatitis Virus C (HCV) infection is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. HCV is an enveloped positive-stranded RNA virus, belonging to the genus Hepacivirus of the Flaviviridae family. HCV depends solely on the host-cell translation machinery for the production of viral proteins. The HCV genome serves as a monocistronic transcript encoding a polyprotein precursor which is processed by proteases and yields at least 10 proteins. An internal ribosome entry site (IRES) residing in the 5′ non-translated region controls translation initiation. Unexpectedly, HCV possesses a second functional ORF within the core region (+1 frame) of the polyprotein encoding an additional protein, known as core + 1/ARFP. Up to date, core + 1/ARFP expression has been studied in transfected hepatoma cells on in an *in vitro* translation system. Multiple forms of the protein, as well as two alternative mechanisms directing its production have been reported: ribosomal frameshift and internal translation initiation. In order to investigate the molecular mechanism controlling core + 1/ARFP synthesis in a more physiologically relevant system, the HCV replicons were used combined with luciferase-tagging experiments and site-directed mutagenesis studies. Bicistronic reporter HCV replicons with Firefly luciferase cloned in frame with core or core + 1/ARF sequence were constructed and introduced into Huh7-Lunet cells. Time-course experiments up to 96 hours revealed that

core + 1/ARFP expression has a different kinetics from the IRES-driven core expression and is more efficient at late time-points. A series of non-sense insertion mutations within the core + 1/ARF ORF designed to evaluate most proposed translation initiation sites indicate the synthesis of at least two different forms of core + 1/ARFP. In addition, a second series of non-sense insertion mutations designed to assess whether core + 1/ARFP expression is independent of the polyprotein expression and the HCV IRES have been constructed and are currently analysed.

P22-8
Regulation of expression of bacterial str-operon through RNA structure and RNA–protein interactions

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Ribosome and its biogenesis are targets in antimicrobial drug development. We report survey of RNA structures responsible for regulation of expression of streptomycin (*str*) operon of bacteria including *E. coli*, *V. cholera*, *M. tuberculosis*. Ribosomal (r-) biogenesis in prokaryotes is driven and regulated by number of RNA-protein interactions. Stoichiometry of rRNA and r-protein level is coordinated through feed-back mechanism: key assembly r-proteins are translational repressors. If rRNA synthesis is high, r-proteins participate in assembly. If rRNA synthesis slows down, excess of r-proteins interact with presumably the same/like RNA motifs on mRNA and stops r-protein translation. For ribosomal small subunit most important key assembly/regulatory proteins are S4 and S7. S7 is responsible for late 3′-end domain assembly; and also regulates translation of *str*-operon. Several approaches have been applied to find similarities in RNA structures recognized by S7. Philogenetic analysis, cross-linking, RNA truncated analysis, selection of combinatorial RNA libraries, computer modeling yielded 3D model of RNA-protein regulatory complex. Two small noncoding RNAs (ncRNAs) were identified which overlap with *str*-operon (out of total 28 intergenical sense ncRNAs in 14 operons and 13 *cis*-antisense ncRNAs in 9 operons). ncRNA were validated by Northern blot to account for growth-stage specific transcription. Internal initiation of transcription was checked throughout bacterial growth by real-time PCR. Novel facets of *str*-operons regulation have been described. Work was supported by NGFNIII 01GS0808, DFG BE 2546/1-2, BMBF 01KI1009A, Malaysia MSTI Nat. E-Sci. Fund 02-01-05-SF0156, RFBR 11-04-01990-a, DAAD A/10/85182.

P22-9
New insights gained from neuronal sub-cellular transcriptomics by deep sequencing

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RNA localization and local translation are regulatory mechanisms of gene expression, conserved from bacteria to mammals. There are several reasons why localized RNA is beneficial to the cell. First, as small amount of RNA molecules can be translated into many copies of a protein, RNA localization can be more economic then the localization of the protein itself. In addition, local translation can ensure rapid localized response to environmental cues. Finally, it enables the cell to overcome the complex transport of proteins that might be toxic when expressed in the wrong place or time.

Although much work has been done in the field of RNA localization, the logic and the principles that underlie the distribution of RNA in the cell are still largely unknown.

In order to approach this problem, we have set up a novel organ culture system that enables the isolation of mRNAs from axons of mouse embryo sensory neurons and combined it with high throughput sequencing (HTS) technology. This allowed us, for the first time, to sequence the whole transcriptom of a sub-cellular compartment – the axon. Analysis of these transcriptoms revealed some unexpected qualities of the axonal RNAs. mRNAs encoding membrane related proteins, many of which are known to act in the axon tip, are poorly represented in the axons. The absence or presence of mRNAs from this group in axons correlate well with the number of membrane spanning domains of the encoded protein, reflecting on the translation and post-translational abilities of the axonal compartment.

Interestingly, while mRNA content seems to differ between axons and cell bodies, miRNAs seems to distribute evenly all over the cell, with five miRs corresponding to more then 70% of total miR expression in these axons.

Finally, using a new bioinformatics approach we discovered short sequence motifs that are enriched in the axons mRNAs.

Overall, this work provides the first sequenced transcriptom of a subcellular domain and shed new light on the logic and the regulation of RNA localization.

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P22-10
Small RNA pathways in Bombyx mori: an *in vitro* and *in vivo* approach

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Lepidopteran insects present high variability in the systemic RNA interference (RNAi) response *in vivo*, which is assumed to depend on as yet not clearly defined species-, tissue- or gene-related factors. In *B. mori*, experiments that involved injection of dsRNAs targeting immune system or sex pheromone gland genes were reported to achieve efficient gene silencing, while RNAi experiments for genes that regulate molting and metamorphosis have shown variable success.

To determine the limiting factors for successful RNAi application in *B. mori*, we used two parallel approaches. First, *in vitro* experiments aiming at the silencing of the key genes that constitute the core RNAi machinery were conducted on the silkmoth-derived Bm5 cell line. Results show that all three small RNA pathways are probably involved in the silencing procedure, as knockdown of Ago1 (miRNA), Ago2/Dcr2 (siRNA) and Ago3 (piRNA) caused significant inhibition of the RNAi process in these cells.

Moreover, a comparative gene expression study of the three key factors constituting the siRNA pathway in the silkworm between different strains, tissues and developmental stages *in vivo*, revealed that Ago2 and Dcr2 are generally more abundant in the P50 than the Daizo strain, while R2D2 expression is maintained at minimal levels in both strains. Interestingly, Ago2 and Dcr2 are upregulated in the midgut compared to the epidermis during the period between the 4th larval molting and pupation, suggesting that the midgut may play an important role in the defense against exogenous dsRNAs (e.g. viral dsRNA).

P22-11
Probing the *in vivo* transcription initiation dynamics of unidirectional promoters at the single molecule level

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The process of transcription initiation in prokaryotes is regulated by multiple factors such as regulatory molecules; RNA polymerase as well as the sequence of the promoter, ionic strength and the structural properties of this region of the DNA sequence. Initiation of transcription in *Escherichia coli* consists of several sequential steps such as formation of the closed complex (RPc); isomerization (RPi), open complex formation (RPo); initiation complex (RPint); and promoter clearance (RPcl). *In vitro* kinetics studies of a *lac* variant promoter confirmed that, in this promoter, there are at least three rate-limiting steps (RLS), namely, RPc, RPi and RPo.

It is unknown to what extent the dynamics of RNA production is determined by the kinetics of these steps in different promoters. Here, we report the results of new measurements that, combined with statistical analysis tools, allow the characterization of the kinetics of several unidirectional promoters. Using an RNA–protein interaction method (MS2-GFP), we measured the time intervals between consecutive transcription events in individual cells under different induction conditions. By fitting an n-step stochastic model to the measured distributions of time intervals we then infer, using maximum likelihood, the number and durations of the RLS for each induction condition. We find that the RLS of the promoters analyzed vary in kinetics depending on the sequence of the promoters and the repression mechanism, suggesting that transcription initiation can have multiple, complex effects on the *in vivo* dynamics of transcription in bacteria. These results provide strong evidence that the RLS, whose kinetics depends on the inducers, determine the mean and fluctuations of RNA numbers of the downstream gene.

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P22-12
Antisense RNA-induced exon-skipping for the gene therapy of frontotemporal dementia and parkinsonism associated with chromosome 17 (FTDP-17)

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A number of neurodegenerative diseases, including FTDP-17 (rare autosomal dominant condition), are characterized by intraneuronal accumulation of the tau protein. The cause of about half of the cases of FTDP-17 are mutations affecting the alternative splicing of exon 10 (E10). The aberrant inclusion of E10 in tau mRNA leads to the aggregation of tau as Neurofibrillary Tangles (NFTs) in neurons.

The project explores the feasibility of an antisense (as-) RNA-based gene therapy to correct tau splicing in FTDP-17. We first tested whether it was possible to modulate E10 alternative splicing by the usage of an Antisense Oligonucleotides (AONs) that masks specific sites regulating splicing pathways. Real Time-PCR and Western blot analyses showed that the transfection of specific AONs is able to alter the splicing behaviour of tau E10 in the rat endogenous transcript (PC-12, rat pheochromocytoma cell lines), with variable efficiencies depending on the concentration of the AONs and on the targeted sequence. These results were confirmed by transfection of Scramble Control oligonucleotides. Based on these results we constructed Adeno-Associated Viral (AAV) vectors coding for specific as-RNAs. We embedded the as-RNA sequences in chimeric U snRNA vectors whose promoters themselves lead to long-term as-RNA expression. We tested whether, the splicing behaviour of tau is corrected in endogenous rat mRNA is corrected by these chimeric antisense snRNAs. To evaluate the effects of AONs/Chimeric Antisense-snRNA on the human tau pre-mRNA, we constructed a minigene reporter system, containing luciferase, we carried out co-transfection into HeLa cells and evaluated the induction of E10 skipping by luciferase expression assay and RT-PCR. Further work will be directed to test the therapeutic efficacy of the AAV-vectored as-RNAs in the animal model of FTDP-17 (T-279 mouse).

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P22-13
The fission yeast *Schizosaccharomyces pombe* possesses two tRNase Z genes involved in the nuclear and mitochondrial tRNA 3′-end processing, respectively

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tRNase Z is the endonuclease that is responsible for the 3′-end processing of tRNA precursors, a process essential for tRNA 3′-CCA addition and subsequent tRNA aminoacylation. In the majority of eukaryotic species, the nuclear and mitochondrial tRNase Z activity is resided in one gene. In contrast, the fission yeast *Schizosaccharomyces pombe* possesses two candidate tRNase Z genes. We show that the two *S. pombe* tRNase Z proteins possess tRNA 3′-endonucleolytic activity *in vitro*. We further demonstrate that these two proteins are involved in the nuclear and mitochondrial tRNA 3′-end processing, respectively. Our results are consistent with partitioning of tRNase Z function between two different proteins in fission yeast. We also discuss why unlike other eukaryotes, *S. pombe* needs two tRNase Z genes.

P22-14
RNA-seq for detailed analysis of *Mycobacterium avium* transcriptome

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Nowadays genomes of multiple bacteria species have been sequenced. Nevertheless, our understanding of bacterial transcriptome is far from complete. Strand-specific RNA-seq is a powerful approach to investigate bacterial transcriptome with unprecedented accuracy. We studied transcriptome of conditionally pathogenic bacterium *Mycobacterium avium* in a mid-log growth phase in culture. Twenty million reads (25–75 nt long) were mapped to *Mycobacterium avium* 104 genome. The data obtained allowed us to reveal 100 candidate cis-encoded small RNAs and 12 trans-encoded small RNAs. Interestingly, trans-

encoded sRNAs are transcribed at a much higher level than cis-encoded, which may indicate different roles playing by these RNAs. Among 12 candidate trans-encoded sRNAs revealed in *M. avium* 7 have homologs in *M. tuberculosis*. sRNA located between genes MAV_1034 and MAV_1035 is of a special interest, because it is expressed at a very high level and has no homolog in *M. tuberculosis*. We determined transcription levels for all annotated genes. Genes coding for ribosomal proteins, transcription and translation factors and DNA-dependent RNA polymerase are transcribed at the highest level, which is consistent with intensive protein synthesis and frequent cell division. Abrupt increases of transcription levels near 5′ ends of many genes were considered putative transcription start sites (TSS). Eight hundred and sixty-two putative TSS were mapped, ten of which were confirmed by 5′-RACE. Thirty-five percent of the identified TSS roughly coincides with start codons. Part of *M. avium* genes has higher level of expression at their 5′ ends compared to the genes bodies, which may indicate either extensive attenuation of transcription or degradation of transcripts.

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P22r-15
Identification of regulatory small non-coding RNAs in *Burkholderia cenocepacia* J2315

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The *Burkholderia cepacia* complex (Bcc) is a group of closely related bacteria that have emerged as important pathogens to cystic Fibrosis (CF) patiens [1]. Although several virulence factors have been identified in Bcc, the knowledge of their relative contribution to global pathogenecity remains scarce. Our research group has recently shown that these bacteria harbor 2 distinct and functional *hfq*-like genes, both required for full virulence [2] [3]. In bacteria, Hfq proteins are global regulators of metabolism, acting as RNA chaperone involved in the riboregulation of target mRNAs by small regulatory non-coding RNAs (sRNAs). Hfq proteins have also been shown recently to interact functionally with sRNAs involved in virulence, including the control of toxin production, biofilm formation, quorum-sensing regulators, and type III secretion system, among others [4]. *Burkholderia cenocepacia* J2315 is a highly virulent clinical isolate encoding 2 Hfq-like proteins in their genome, both required for optimal survival to stress and full virulence [2] [3].

Based on co-purification experiments using his-tagged Hfq and Hfq2 and small sized fractions of total RNA were extracted from *Burkholderia cenocepacia* J2315, we have recovered about 50 putative sRNAs. Northern Blots are being performed to confirm the expression of these putative sRNAs, time whereas bioinformatics analysis is being performed in order to predict possible mRNA target to orientate experimental work aiming at the understanding of their physiological role in the context of infection.

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P22-16
Preliminary biochemical and functional analysis of *hDIS3* mutations associated with pathogenesis of multiple myeloma

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Multiple myeloma (MM) is a lethal neoplastic disease which accounts for over 1% of all tumors, 10–15% of haematological malignancies, and 20% of deaths related to blood cancers with the incidence rate of 4–5 individuals per 100 000 per year. Recent sequencing of cancer cells form 38 MM patients led to the unexpected finding that besides the well known activating mutations of the Ras proto-oncogene, the most common were in the *hDIS3* gene.

hDIS3 encodes one of the catalytic subunits of the major eukaryotic ribonucleolytic complex – the RNA exosome. *hDIS3* is a multidomain protein endowed with two different enzymatic activities: exonucleolytic, located in the RNB domain, and endonucleolytic, originating from the N-terminal PIN domain. Mutations found in MM are highly specific towards the RNB domain and one of them is in the identical position to the previously identified suppressor mutation in yeast, which does not inactivate Dis3 completely but rather weakens or modifies the exonucleolytic activity. As the exosome is involved in virtually all processes associated with eukaryotic RNA turnover, it can be expected that *hDIS3* mutations detected in MM may have pleiotropic effects on RNA metabolism.

The aim of our research was the initial analysis of these mutations. Results of *in vitro* biochemical assays indicate that some of them lead to various disturbances in degradation of single- and double-stranded RNA molecules. Moreover, when corresponding mutations were introduced in yeast, it resulted in cell-growth inhibition or temperature-sensitivity phenotype, accompanied by accumulation of the known physiological exosome substrates. Preliminary studies on human cell lines bearing those mutations also demonstrated that they adversely affect growth of the cells. Overall, our results suggest that mutations found in MM indeed influence the cell physiology and exosome function.

P22-17
Abundance sensitive diferentially expressed cluster detection for RIP-Seq

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With the advent of massively parallel high-throughput sequencing of short DNA fragments, RIP-Seq has become a highly attractive method for detecting binding sites of RNA-binding proteins (RBPs) or protein complexes on a genome-wide scale. It involves immunoprecipitation of RNA-protein complexes using antibodies against target protein/s of interest followed by detection of RNA binding sites by high-throughput sequencing. Millions of reads obtained are analyzed using computational tools to reveal the binding sites of RBPs. Any computational pipeline for analysis of high-throughput sequencing data to reveal binding sites of RBPs faces a challenge to minimize noise that arises from experimental methods, sequencing quality or mapability. Furthermore, background noise is a direct function of expression levels of individual genes. To account for these issues, we have developed a

new cluster (peak) detection algorithm that computes a background for any expressed exon using its expression levels. Such background definitions allow us to robustly detect peaks even in genes with low expression levels. Moreover, to understand the regulation mechanism of RBPs and their targets in the certain conditions, differentially expressed sites of the RNA footprints aim to be detected in between two different experimental conditions. According to the designed experiment, the conditions can be stressed induced cells versus wild type or any other two conditions want to be explored. We have used this algorithm to detect clusters in two different fields. First one is based on detection of genome-wide binding sites of a multi-protein complex (Exon Junction Complex, EJC) that is deposited on mRNA 20–24 nucleotides upstream of exon-exon junctions during splicing of pre-mRNAs by tandem purification of two proteins in the complex with two different protein sets. By combining EJC footprint data with an RNAseq library from the same cells to measure relative mRNA abundance in HEK293 cells, we are finding that EJC occupies regions centered ~24 nucleotides upstream of exon-exon junctions on most human mRNAs. Moreover, there are other secondary non-exon junction peaks that may represent binding sites of EJC interacting proteins. We also investigated the differences between two different protein pull downs to understand the roles of the functions of different proteins in EJC complex. In the second project, our algorithm is used to detect Staufen binding targets by comparing WT versus mutated version of Staufen in HEK293 cells. We will present these and other findings with an emphasis on computational strategies and tools utilized to reveal genome-wide RIP-Seq analysis.

P22-18
The mechanism of alternative splicing of the X-linked NDUFB11 gene of the respiratory chain complex I, impact of rotenone treatment in neuroblastoma cells

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Genes use alternative splicing as part of their expression program. Changes in the alternative splice isoforms ratios have been associated with physiological variation and susceptibility to disease. Pre-mRNAs exhibit cis-acting elements, exonic splicing enhancers (ESEs) and exonic splicing silencers (ESSs), which are recognized by two protein families: heterogeneous nuclear ribonucleic particles proteins (hnRNP) and the Serine/Arginine rich protein family. The competition between ESEs and ESSs ensures that only authentic splice sites are used. This enables, in addition, a regulated usage of weak splice sites. Recent studies have shown that rotenone, a complex I inhibitor, alters splicing of different mRNAs in neuroblastoma cells. Alteration in the expression of parkin splice variants, in sporadic Parkinson disease and in dementia with Lewy bodies, has been demonstrated, suggesting that there may be problems with mRNA splicing in neurodegenerative diseases associated with mitochondrial complex I dysfunction. A study is presented on the regulation of alternative splicing (AS) of the Ndufb11 gene of the mitochondrial complex I and the impact on this process of rotenone in neuroblastoma cells. Physiologically the Ndufb11 gene produces at high level a short transcript isoform encoding for a 153 aa protein. This subunit is essential for a functional mammalian complex I. The gene produces also, at low level, a longer transcript isoform encoding for a 163 aa protein whose role is unknown. We show here that the level of the two isoforms is regulated by DGGGD ESS elements located in exon 2 which bind the hnRNPH1 protein. Rotenone affects the Ndufb11 alternative splicing, with increase of the 163/153 mRNAs ratio. This appears to be due to down-regulation of the hnRNPH1 protein.

P23 – Single Molecules

P23-1
Synthesis and evaluation of the efficiency of RND type efflux pump inhibitors in bacteria

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Salmonella enterica serovar Typhimurium is the main cause of food poisoning and is the second most common cause of bacterial diarrhea [1-2]. Resistance-nodulation-cell division (RND)-type multidrug resistance efflux pumps are the main reason of *Salmonella enterica* cell resistance to antibiotics. Inhibitors of drug efflux pumps have great potential as pharmacological agents that restore the drug susceptibility of multidrug resistant bacterial pathogens [3].

RND-family drug efflux pumps are often associated with the recalcitrance of Gram-negative bacteria to antibacterial drugs, it is very important to discover molecules that inhibit these pumps and to investigate the mechanism of efflux pump inhibition.

The aim of our work was to synthesize small dipeptide inhibitors and to evaluate the efficiency of RND-type efflux pump inhibition in *S. enterica* cells.

In this work we present data on the electrochemical assay of accumulation of an indicator compound (TPP^+) of efflux pumps in bacteria. We studied effects of the synthesized RND-type efflux pump inhibitors on the accumulation of TPP^+ ions in *S. enterica*. Data on the effective concentrations of the inhibitors and the efficiency of inhibition of RND-type efflux pump activity as well as synthesis of the inhibitors will be presented.

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P23-2
The Interaction of DNA with linker histones

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Spermatogenesis is accompanied by various physical and biochemical changes in the nucleus. First of all, there come changes of the protein environment of DNA. In particular, H1 histones are replaced by sperm-specific variants. Sperm-specific proteins are characterized by a long polypeptide chain, and increased arginine content in comparison with somatic histone. These peculiarities determine specific DNA compaction degree. We have investigated structural features of the complexes of DNA with histone H1 isolated from the sperm of marine invertebrates: sea urchin, starfish and bivalve by the methods of UV spectroscopy, circular dichroism (CD) and determination of DNA melting temperature. Comparison of the CD spectra of complexes of DNA with histones H1 spermatozoa of animals belonging to different taxa, showed that all studied sperm-specific histones do not compact DNA in low ionic strength solutions. At physiological conditions H1 from sea urchin and starfish sperms compact DNA more intensively than other histones. Our previous detected additional α-helical regions in the C-terminal domain of H1 spermatozoa from echinoderms facilitate protein–protein interactions and stimulate cooperative interaction of proteins with DNA. Here we have received DNA thermodynamic characteristics. We found that mollusc sperm histone H1 stabilizes DNA more than other proteins. Echinoderm sperm histones H1 take an intermediate position with regard to both melting temperature and the ratio of free and bound to the protein DNA. We suppose that there is no a single mechanism for supercompact sperm chromatin formation, and the DNA compaction mechanism in the nucleus depends primarily on the characteristics of the structural organization of proteins.

P23-3
Using optical tweezers to study the DNA unwinding dynamics of a processive DNA polymerase

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Duplication of double-stranded DNA (dsDNA) requires a fine-tuned coordination between the DNA replication and unwinding reactions. Using optical tweezers we probed the coupling dynamics between these two activities when they are simultaneously carried out by individual Phi29 DNA polymerase molecules replicating a dsDNA hairpin. We used the wild-type and an unwinding deficient polymerase variant and found that mechanical tension applied on the DNA and the DNA sequence modulate in different ways the replication, unwinding rates and pause kinetics of each polymerase. However, incorporation of pause kinetics in a model to quantify the unwinding reaction reveals that both polymerases destabilize the fork with the same active mechanism and offers insights into the topological strategies that could be used by the Phi29 DNA polymerase and other DNA replication systems to couple unwinding and replication reactions.

P23-4
Deciphering repetitive DNA methylation patterns on single DNA fibers using Epi-Combing

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In vitro methylated and hydroxymethylated DNA and then applied for the characterization of higher-ordered arrangements of genomic DNA modifications. Epi-combing of ribosomal DNA (rDNA) in combination with immuno-FISH experiments reveal the clustering of transcriptionally active and inactive rDNA copies in the linear genome, furthermore it shows the stable DNA methylation of non-functional rDNA repeats. Our study initiates the locus-specific analysis of repetitive DNA modifications and

opens new way for epigenomic biomarker discovery and epigenetics-based diagnostics.

P23-5
Single-molecule FRET measurements in bacterial cells

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Most single-molecule FRET experiments are performed *in vitro*, using tightly controlled conditions and well-defined concentrations of a limited number of interacting components. However, in order to understand biological mechanisms as they occur *in vivo* while taking advantage of the extra information provided by single-molecule detection, there is a growing need for performing single-molecule fluorescence measurements in cellular contexts, and in particular in living cells. Controlled internalization through microinjection of proteins labelled with an organic FRET dye pair allowed single-molecule FRET observation in living eukaryotic cells (Sakon & Weninger, 2010). However, such a technique cannot be applied to bacteria due to their small size relative to the microinjection syringe.

We have developed physical methods for delivering fluorescent biomolecules in living *Escherichia coli* and observing single-molecule fluorescence and FRET in the bacterial cytoplasm; we use confocal, total-internal-reflection fluorescence and wide-field imaging approaches for detection, providing access to a large number of probed timescales. We are also able to tune the concentration of internalized material from high concentrations compatible with super-resolution imaging to lower concentration compatible with single-molecule observation. Our results using single-stranded and double-stranded DNA standards with different FRET efficiencies show that the FRET efficiency of the internalized DNAs agrees well with *in vitro* FRET measurements. Single-molecule FRET time-traces from the majority of internalized molecule show the characteristic spectroscopic signatures expected from a single FRET pair system. Ongoing work on other biomolecules, including doubly labelled proteins, should lead to the exciting prospect of visualizing sub-nanometre conformational changes at the single-molecule level in the natural milieu of live cells. Our approaches are general and should be useful for studying a large number of intracellular processes in bacteria.

P23r-6
Nanomechanics of ferredoxin-NADP$^+$ reductase complexes analyzed by AFM single-molecule force spectroscopy

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Atomic Force Microscopy (AFM) is the only technique capable to study the morphology and properties of biosystems in a physiological environment with nanometer resolution. Force Spectroscopy measures the mechanical forces between the AFM tip and a sample, allowing analyzing the forces between a ligand attached to the tip and a receptor bound to the sample. Here, the first measurements of the intermolecular forces of flavoproteins complexes are presented. During photosynthesis, Ferredoxin-NADP$^+$ reductase (FNR) catalyses the electron transfer from Ferredoxin (Fd), or Flavodoxin (Fld), to NADP$^+$. This process requires the formation of optimally oriented transitory competent complexes for electron transfer. A strategy has been developed to immobilize the proteins without altering their functionality and surface interactions. This methodology allowed to achieve a high efficiency in the formation of bonds over that of approaches, 61% for Fd and 77% for Fld, applying forces of 0.34 and 0.22 nN, respectively. These results can be interpreted as a consequence of the more specific interaction described in the FNR:Fd complex versus the existence of alternative binding modes contributing to the electron transfer in the FNR:Fld complex, as well as of the smaller interaction interface in the Fld complex. Rupture forces of 21 ± 1 and 57 ± 1 pN for Fld and Fd complexes were obtained respectively at a loading rate of 10 nN/sec indicating a greater mechanostability for the complex with the natural electron donor when iron is available.

P23m-7
T cell receptor analyzed with super-resolution imaging secondary ion mass spectrometry

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The engagement of the T cell antigen receptor (TCR) by a specific peptide-MHC ligand initiates an tran-membrane signaling to activate T cell. An important event in T cell signaling is the recruitment of signaling proteins to the cell membrane and the assembly of a multi-protein signaling complex. Cytoskeleton-dependent clusters are formed immediately after TCR engagement and move to the center of T cell-antigen presenting cell interface to form the immunological synapse. But a mechanistic understanding on the T cell membrane dynamics and its implication for TCR-mediated signaling is still lacking. Here, we characterize the T cell receptor and other signaling components during T cell activation by super-resolution secondary ion mass spectrometry (NanoSIMS) with a lateral resolution of ~50 nanometers. Quantitative information about the chemical composition within T cell membrane domains was obtained with the use of isotopic labels to identify each signaling molecules. We found that TCRs accumulate at 60–100 nm clusters on the activating T cell membrane. Because these membrane dynamic structures are shared with many other cell types, we believe that the insights we gain in this system will be generalizable to other examples of cell surface receptor signaling.

P23-8
Atomic force microscopy reveals a dimer of trimers organization in *Corynebacterium ammoniagenes* FAD synthetase

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All organisms transform riboflavin, first into FMN, and then into FAD, by the sequential action of two activities, *ATP:riboflavin kinase* and *ATP:FMN adenylyltransferase*. However, whereas eukaryotes use two enzymes for *FMN* and *FAD* production, most prokaryotes depend on a single bifunctional enzyme, the *FAD synthetase* (FADS). Due to the differential molecular

characteristics of both types of enzymes, inhibition of prokaryotic *FADS* might be a feasible treatment for pathogenic diseases. Using as model the *FADS* from *Corynebacterium ammoniagenes* (*CaFADS*), binding and catalytic parameters for the substrates at the two catalytic sites, and the presence of one *ATP* and one flavin binding site at each of the catalytic sites were determined and the 3-D structure solved. In the present work we have used *Atomic Force Microscopy* (*AFM*), the only microscopic approach that can measure the topography of biological systems in aqueous media with nanometer resolution, to clarify the factors inducing the formation, stoichiometry and putative functional role of the *CaFADS* oligomers detected in solution. The enzymatic samples were imaged mimicking the intracellular conditions. *AFM* has allowed us not only to observe the topology of single molecules, even identifying each domain, but also to identify how the presence of particular ligands induces the formation of the dimer of trimers reported for its crystal structure. Additionally, we also show detection of these oligomeric species in *C. ammoniagenes* and *E. coli* cultures overexpressing *CaFADS*. The results suggest that oligomeric transient complexes of *CaFADS* must be produced during the catalytic cycles of this enzyme and that the quaternary organization might contribute to regulate flavin homeostasis within the cell.

P23-9
YY1 is a strong activator of SDF-1 gene expression

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Type 1 diabetes (T1D) is a multifactorial disease characterized by hypoinsulinemia caused by a deficiency of pancreatic beta-cells. It has been shown that SDF-1 promotes pancreatic beta-cell survival via activation of the prosurvival kinase Akt. Since factors involved in the control of beta-cell growth and survival could provide new approaches for the treatment of T1D in its early stages, we initiated the investigation of molecular mechanisms that regulate gene expression of SDF-1, a potential beta-cell growth factor.

Using the ALGGEN PROMO database, we identified in the SDF-1 promoter six putative binding sites for YY1. Super-shift analysis revealed that YY1 was present in the nucleoprotein complex formed between the SDF-1 promoter and pancreatic beta-cell nuclear proteins. *In vivo* chromatin immunoprecipitation (ChIP) analysis confirmed these results. Two predicted YY1 motifs in the SDF-1 promoter overlap with the potential Kozak sequence suggesting that the putative translational start site is superimposed with YY1 motif. Functional analysis using luciferase assay showed significantly enhanced SDF-1 promoter activity when YY1 was overexpressed. These data suggest that YY1 upregulates SDF-1 promoter activity and therefore SDF-1 gene transcription *in vivo*.

This study showed for the first time that YY1 is able to bind the SDF-1 promoter. Furthermore, our investigation demonstrated strong activation of the SDF-1 promoter by YY1, suggesting that YY1 is an important transcriptional activator of the SDF-1 gene.

P23-10
A small cold shock protein as a model for force spectroscopy studies of extremophilic proteins

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Life has adapted to a multitude of different environmental conditions to the extent that it is difficult to find place on Earth, which is devoid of living beings. Some conditions are unfavourable to most eukaryotes and are considered extreme. To colonise extreme ecological niches requires the adaptation of proteins. Understanding the remarkable physical properties of proteins from extremophiles is of fundamental interest and invaluable for the *de novo* design of proteins with desired properties for biotechnological and medical exploitation.

We have built a force spectroscopy instrument to examine the conformational dynamics of single extremophilic proteins. This technique applies a stretching force along the end-to-end length of a protein, driving proteins to a fully extended unfolded state. By examining single molecules one at a time, the individual dynamics of protein subpopulations can be measured, revealing information which may be crucial for the understanding of how extremophilic proteins are adapted to their specific environment.

For our studies we selected a small cold shock protein (CSP) from *Thermotoga maritima* (*Tm*), a hyperthermophilic bacteria. The protein's five anti-parallel β-strands are arranged in two β-sheets to form a mechanically robust β-barrel structure. CSPs are ancient transcription factors that are evolutionary conserved and present in all branches of life. This provides a range of homologous proteins from extremo- and mesophilic organisms to facilitate comparative studies.

We show that *Tm*CSP requires less force to unfold and is less sensitive to an applied force than the I27 protein, which is used as a control to unambiguously identify the *Tm*Csp signal within force-extension traces. Combined with Monte Carlo simulations, *Tm*Csp shows a higher unfolding rate and a longer distance to the unfolding transition state at zero force than I27. Establishing a basic description of the energy landscape of *Tm*Csp will allow further experiments to explore the effect of different environmental conditions on the folding behaviour of this extremophilic protein.

P23-11
Mapping of *Hantavirus glycoprotein* intraviral tails interaction with ribonucleoprotein

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Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are zoonotic viruses that chronically infect rodents and insectivores causing no apparent disease but when transmitted to man they are linked to two major clinical symptoms: hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. The release of hantaviruses from infected cell requires specific interactions between structural proteins of the virus to take place. Recently, we identified an interaction between intraviral tails of envelope glycoproteins Gn and Gc with the viral nucleoprotein complex (RNP; consisting of N-protein encapsidated RNA), most likely a key event in the egress of the virus from its host cell. We now demonstrate that the intraviral tail of Gn has affinity, not only

towards the N-protein, but also to nucleic acids. In contrast, the cytoplasmic tail of Gc only binds the N-protein. We mapped the interaction of Gn-tail with peptide arrays towards, RNP, N protein and nucleic acids. The relative affinity of the binding sites obtained by peptide mapping was analyzed by soluble peptides and recombinant Gn-tail proteins. Taken together, we identified the binding sites of N-protein and nucleic acids to overlap in large extent and suggest three different binding sites in Gn-tail towards the RNP, of these the most C-terminal peptide stands out as the peptide of highest affinity.

P23-12
Novel amphiphilic compounds inactivate the RNA- and DNA-containing viruses

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The artificial ribonucleases (aRNases, small compounds possessing the ability to cleave RNA *in vitro*) represent a new promising class of antiviral agents: they were shown to inactivate the RNA-containing enveloped influenza A [1] and encephalitis [2] viruses and non-enveloped Acute bee paralysis virus (ABPV) [3] under physiological conditions. Three groups of aRNases were designed recently: compounds which contain catalytic groups for the RNA cleavage (imidazole, arginine) and positively charged 1,4-diazabicyclo[2.2.2]octane (DABCO) substituted at the bridge nitrogens with dodecyl residues; compounds containing two similarly N-substituted DABCO residues, but no catalytic groups; peptide-mimicking compounds containing amino acids as catalytic groups and hydrophobic residues. The inactivation of enveloped viruses was due to the synergetic effect of the viral RNA cleavage and envelope disruption. In case of non-enveloped ABPV the virus inactivation was due to viral RNA cleavage, but the morphology of virus particles was not affected. Here we demonstrate that aRNases exhibite the antiviral activity against DNA-containing vaccinia virus: the screening of aRNases revealed that only amphyphilic aRNases containing positively charged DABCO residues substituted at the bridge nitrogen with aliphatic groups efficiently inactivate the virus. The concentration profile and time course of virus inactivation revealed the basic stages of virus inactivation; the electron microscopy study of the inactivated virus preparation revealed the significant alterations of the viral morphology in the course of inactivation: the surface proteins were disorganized and membrane destruction was visualized. Based on the obtained results, we can conclude that aRNases are universal agents for the inactivation of the viruses of various classes.

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P23-13
Optical absorption of human blood in UV-VIS and NIR

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In the present work we report results of investigations influence of ionization radiation on human blood by optical absorption in ultraviolet (UV), visible (VIS) and near infrared (NIR) region from 200 to 2500 nm. Blood was donated by healthy adult volunters and collected under air in glass tubes containing a small amount of sodium citrate or heparin used as an anticoagulant. Blood without any anticoagulant was also tested and showed no difference with respect to sodium citrate-treated or heparin blood.

The optical absorption spectra of blood were recorded in the energy range from 2500 to 190 nm with the spectral resolution 0.1 nm using the double-beam spectrophotometer Jasco spectrophotometer (Model V-570). Optical absorption spectra of the blood have absorption peaks in UV 276, 340 nm in VIS 412, 541, and 576 nm. Absorption band are present in NIR absorption spectra centred 1690, 1740, 2056, 2170, 2290, 2350 and 2460 nm. After ionization radiation additional absorption bands appear in optical absorption spectra of human blood. We observed the additional absorption bands 630 nm in VIS ragion of absorption spectra and NIR region about 1000 nm.

P23-14
Adenine–thymine interactions on gold electrodes studied by *in-situ* electrochemical FT-IR-spectroscopy

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The DNA complementary bases adenine and thymine are known to play an important role in genetic expression and replication as well as in biological relevant enzymatic processes. The study of the interactions of these complementary bases in organised structures can contribute to the understanding of their physiological activities. On the other hand, it is known that both bases adsorbed on metal substrates forming organised films that have been studied by electrochemical techniques and by *in-situ* vibrational spectroscopies [1] [2] [3]. These kind of studies are also interesting in many biotechnology and nanotechnology applications as for instance in the design and fabrication of biosensors. On gold electrodes both bases form chemisorbed films by interactions through some of the atoms which are also active in DNA interactions [1] [2] [3]. The coadsorption of thymine and adenine at Au(111) has been previously reported as studied by cyclic voltammetry and found to be greatly dependent on the experimental conditions [4].

The combination of electrochemical methods with modern *in-situ* FTIR techniques that provide chemical specificity together with high sensitivity, allow the molecular characterisation of organised structures. In this work cyclic voltammetry is combined with *in-situ* external IR reflection experiments on Au(111) and with internal reflection experiments on gold thin-film electrodes in order to understand the organisation and interactions between the two complementary DNA bases on the metal surface. Experiments have been performed in $KClO_4$ solutions of different pH values at different DNA bases concentrations. The FTIR spectroscopy measurements have been performed using H_2O and D_2O as solvents.

It has been observed that the behaviour greatly depend on the applied potential and the ratio between the adenine and thymine concentrations. At low potentials, at which thymine is physically adsorbed, chemically adsorbed adenine replaces thymine from the surface even when thymine concentration is about 100 higher than adenine concentration. However, co-adsorption of the two bases is detected at high potentials at which thymine get chemically adsorbed. The conclusions are reached by comparing the spectra of the co-adsorbed species with the spectra of the individual bases at the different potentials applied to the electrode surface.

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P23-15
Mechanisms of HSA adsorption on mica: electrokinetic studies

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Adsorption of HSA from aqueous solutions on mica was studied using AFM, *in situ* streaming potential measurements and XPS. In our study, determining bioparticles adsorption mechanisms, emphasis was focused on developing an efficient procedure of a direct, *in situ* determination of protein coverage via the streaming potential measurements. Bulk physicochemical properties of HSA and the mica substrate were characterized for various ionic strength and pH. The kinetics of HSA adsorption under diffusion-controlled transport was quantitatively evaluated using AFM for coverage up to 20%. For higher coverage, adsorption was determined via streaming potential measurements performed using a parallel-plate, microfluidic cell. It was shown that HSA adsorption was irreversible. These experimental results were consistent with AFM measurements and with theoretical predictions based on the random sequential adsorption model. A quantitative relationship between the amount of adsorbed protein, zeta potential of the interface and the particle coverage are determined in terms of the theoretical model postulating the effective hard particle concept previously used for colloid particles. Adsorption of HSA for the higher coverage range was also studied by XPS. It was established that the maximum coverage of irreversibly bound HSA on mica at pH = 3.5 was close to 1.6 mg/m^2 in accordance with streaming potential measurements. From these experimental data it was concluded that HSA adsorption on mica at various pH proceeded under electrostatically controlled mechanisms with no tendency to multilayer formation. Results proved that the coverage of HSA can be quantitatively determined using the streaming potential method, especially for $\Theta < 0.2$, where other experimental methods become less accurate. Such monolayers of HSA of well- defined coverage and molecule orientation can find various practical applications, e.g., for performing efficient immunological tests, designing implanted biomaterials surface and biosensors.

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P23-16
Watching and manipulating single molecules involved in DNA repair

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Proper development of life relies on multiple DNA transactions that occur in the nucleus of the cell including repair and maintenance of its genetic material. DNA manipulations are performed by proteins acting as truly nano-machines cutting, pasting, or copying fragments of DNA in a highly efficient manner. Development of techniques to allow manipulation and visualization of nucleic acids at the single molecule level can help understanding how these complex transactions are performed. Single molecule approaches allow observation of reaction intermediates and grant access to previously unobtainable data of fundamental biochemical processes. In this talk, I will show how the atomic force microscope and single-molecule tweezers techniques can watch and manipulate protein machines in their function to repair DNA. In particular, I will focus on two particular systems: SMC proteins involved in the maintenance of the structure of chromosomes and, AddAB, a helicase-nuclease responsible for the preparation of DNA ends to undertake repair by Homologous Recombination.

P23-17
KfrA protein of R751 plasmid study by DLS, microelectrophoresis and AFM method

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Recombinant KfrA is a DNA binding protein, involved in survival of IncP-1 plasmids R751. Physicochemical properties in the bulk were determined using Malvern Zetasizer Nano. The average size of KfrA protein (hydrodynamic diameter) determined by dynamic light scattering (DLS) and AFM, was 10–12 nm (pH = 3.5, I = 10^{-3}M). It was also revealed from these measurements that the protein exists in an aggregated stated, probably composed of four molecules. Physicochemical properties and adsorption mechanisms of recombinant KfrA protein on mica were thoroughly characterized using atomic force microscopy (AFM). KfrA were deposited directly on freshly cleaved mica sheets. The protein coverage was determined as a function of time and bulk protein concentration. It was also confirmed that KfrA adsorption on mica was irreversible for pH = 3.5. These results were in agreement with theoretical predictions pertinent to diffusion-controlled transport. Afterwards, the measurements of the electrophoretic mobility of KfrA-covered latex were carried out as a function of the amount of adsorbed protein, expressed as the surface concentration. In the experiments the polystyrene latex particles 800 nm in diameter were used. The zeta potential of KfrA-covered latex was determined by microelectrophoresis measurements. The dependence of zeta potential on the bulk concentration of protein was investigated. These results were used as reference data to determine the lowest concentration of the protein needed to form the KfrA monolayer on latex particles. The dependence of the mobility and the zeta potential of protein-covered latex on pH was also investigated. The zeta potential of protein-covered latex was positive for pH lower than 4.5 (isoelectric point) and negative otherwise for the ionic strength of 10^{-3} M NaCl. It was revealed that KfrA molecules exhibited a tetramer structure. Moreover, our experimental data delivered of physicochemical properties of KfrA protein are of importance to explain mechanisms and site binding of DNA. Acknowledgment:

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P23-18
EPR method to analyze radiation effects in human blood

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In the present work we report results of investigations of human blood after irradiation during radio-isotopes (Tc_{99m}) and intravascular diagnostics by electron paramagnetic resonance (EPR). Blood was donated by consenting patients before and after diagnosis and collected under air in glass tubes containing a small amount of the anticoagulant. The EPR spectra were recorded using an EMX-6/1 spectrometer (BRUKER) working at X-band frequency with 100 kHz modulation. Magnetic field varied between 60 and 7000 Gauss. The g-factors of EPR signals were determined by reference to the external magnetic field. The EPR signal intensities in blood were measured against fixed standard signals. The EPR spectra of blood have been studied at 80 K temperature. It is shown that EPR spectra of blood of patients after examination by radio-isotopes diagnosis has signal of the ion Fe^{3+}. of methemaglobin is in low-spin state with $g = 2.3$ and in the high spin state with $g = 6$. We can also detect EPR signals from the metal-protein transferrin ($g = 4.3$) that contains the non-haem rhombic iron. A method of EPR spectral analysis of quantitative signals was used to perform investigation of the blood before and after diagnosis. The results recieved from double integral values of the individual lines gives us information about concentration of all the paramagnetic centrs present. EPR spectroscopy could to be powerfull instrument for investigation of influance of radiation on human blood.

P24 – Systems Biology

P24-1
Comparison of infection strategies of bacteria and viruses

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Infectious organisms, pathogens cause diseases by interactions with human proteins. The advances in high-throughput protein interaction detection methods have enabled the collection of large-scale data on pathogen–host protein–protein interactions (PHIs). We have developed Pathogen Host Interaction Search Tool (PHISTO) that provides the entirety of relevant information about the PHIs in a single resource. It offers access via a user-friendly and functional web-interface (www.phisto.boun.edu.tr). We have analyzed the most comprehensive available pathogen–human protein interaction data including 23 435 interactions, targeting 5210 human proteins, which were obtained from PHISTO. This is the first comprehensive attempt to get a comparison between bacterial and viral infections. We investigated human proteins that are targeted by bacteria and viruses to provide an overview of common and special infection strategies. We observed that in the human protein network the proteins targeted by pathogens have higher connectivity and betweenness centrality values than those proteins not interacting with pathogens. Compared to bacteria, viruses tend to interact with human proteins of much higher connectivity and centrality values. Gene Ontology enrichment analysis of the human proteins targeted by pathogens indicates crucial clues about the infection mechanisms. As the main infection strategy, bacteria interact with human proteins that function in immune response. Indispensable viral strategy, on the other hand, is the manipulation of human cellular processes. A novel observation about pathogen-human systems is that the human proteins targeted by both pathogens are enriched in the regulation of metabolic processes.

P24-2
A novel strategy for the comprehensive analysis of the biomolecular composition of isolated plasma membranes

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We manufactured a novel type of lipid-coated superparamagnetic nanoparticles that allow for a rapid isolation of plasma membranes (PMs), enabling high-resolution proteomic, glycomic and lipidomic analyses of the cell surface. We used this technology to characterize the effects of presenilin knockout on the PM composition of mouse embryonic fibroblasts. We found that many proteins are selectively downregulated at the cell surface of presenilin knockout cells concomitant with lowered surface levels of cholesterol and certain sphingomyelin species, indicating defects in specific endosomal transport routes to and/or from the cell surface. Snapshots of *N*-glycoproteomics and cell surface glycan profiling further underscored the power and versatility of this novel methodology. Since PM proteins provide many pathologically relevant biomarkers representing two-thirds of the currently used drug targets, this novel technology has great potential for biomedical and pharmaceutical applications.

P24-3
Global transcriptional response of *Saccharomyces cerevisiae* to copper load

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Copper is a trace element which is required for normal growth and the development of bone, brain, immune system and red blood cells during infancy. Copper dependent enzymes are required for mitochondrial respiration, antioxidant defense, neurotransmitter synthesis, connective tissue formation, pigmentation, peptide amidation and iron metabolism. Maintenance of the homeostatic balance to perform these processes is vital since excessive accumulation of copper leads to toxicity. *Saccharomyces cerevisiae* has been used as a model organism to understand

the copper metabolism in human due to its high degree of conservation. *CCC2*, which is the yeast ortholog of Cu-ATPase encoding genes; *ATP7A* and *ATP7B*, encodes a protein, which transfers copper across the membrane into lumen of the Trans Golgi Network. The lack of *CCC2* leads to a defect in the transport of copper to apoFET3 and thus iron deficiency. The yeast cells lacking a functional *CCC2* gene show respiratory deficiency, which can be reversed in copper supplemented medium. In this study, the genome wide transcriptional response of yeast cells to high concentrations of copper in the absence of *CCC2* gene were investigated in comparison to wild type cells. The genes that show significant changes at their transcription levels were further analyzed in order to gain more insight about the related pathways.

P24-4
Cellulose consolidated bioprocessing to biofuels: one goal, two strategies

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Economic viability of biorefinery strategies, producing high added-value molecules by microbial fermentation, relies on the utilization of cheap substrates and processes. Lignocellulose is the most attractive biomass because of its high abundance, low price and reneawability. However, current cellulosic biomass bioconversion processes consist of economically inefficient multistep configurations.

Our research projects aim to develop consolidated bioprocessings for one-step waste cellulose fermentation into biofuels. Two strategies are currently employed: (i) the co-cultivation of a natural cellulolytic bacterium (i.e. *Clostridium cellulovorans*) and the solvent producing *Clostridium acetobutylicum* for ethanol, butanol and hydrogen production; (ii) the construction of a recombinant butanol hyperproducing *C. cellulovorans* by genetic engineering of its metabolic pathways.

Hydrogen and butanol production were obtained by *clostridium* co-cultivation approach. Current studies are aimed to enhance biofuel production by this approach by identifying the metabolic bottlenecks that limit either cellulose hydrolysis and/or soluble carbohydrate conversion into metabolites of interest.

Parallel studies are aimed to set up efficient protocols for *C. cellulovorans* transformation and gene manipulation. A global *C. cellulovorans* metabolism modification, including both rational engineering and *in vivo* evolution, is envisaged so as to obtain a strain showing both high butanol production yield and productivity and enhanced butanol tolerance.

P24-5
Systematic approaches towards drafting the neuronal ceroid lipofuscinoses interactome

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The Neuronal ceroid lipofuscinoses (NCLs) represent the most common cause of inherited progressive encephalopathy of childhood, characterized by accrual of autofluorescent, ceroid-like lipopigment and degeneration of cortical neurons. Despite recent progress in the NCL field, the mechanistic and biological functions of most NCLs remain unclear [1]. Thus, we aim to apply systematic approaches including: functional proteomics, bioinformatics, quantitative co-immunoprecipitation, membrane yeast two-hybrid (MYTH) and mouse disease models, towards drafting the NCL interactome.

In order to systematically investigate the functions of NCLs, we initiated a proteomic analysis of TAP-tagged NCLs and their associated protein complexes, in SH-SY5Y human neuroblastoma cells. Protein complexes were isolated and identified by Tandem affinity purification coupled to mass spectrometry (TAP-MS) [2]. Bioinformatic analyses of isolated complexes, yielded: 34 CLN3 interacting partners including four novel CLN3 IPs, as well as 14 CLN3 HCIPs and 16 LCIPs previously identified by Behrends C, et al. Nature, 2010 [3]. Moreover, 20 CLN5 IPs (12 of which are common with the CLN3 bait) were also identified. To assign confidence values to individual protein–protein interactions, we utilized a statistical platform-Significance Analysis of INTeractonme (SAINT) [4], followed by Gene Ontology, pathway and functional analyses. Our findings support previously suggested involvement of CLN3 in transmembrane transport, lipid/protein metabolism, myelination, neuronal excitability, as well as link it to G-protein signalling and protein folding/sorting in the ER.

In future experiments, we aim to utilize a novel technique amenable to analysis of interactions among hydrophobic proteins, the Membrane Yeast Two- Hybrid assay (MYTH: in collaboration with Prof. Stagljar lab (Donnelly Center, University of Toronto). This will allow us to study binary interactions of several predicted membrane associated NCL proteins (PPT1, TPP1, CLN3, CLN5-CLN8).

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P24-6
The metabolic responses of yeast to two drug agents: rapamycin and caffeine

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Target of rapamycin (TOR) is one of the major regulatory mechanisms in many organisms from yeast to human. Several processes including growth, nutrient metabolism, protein synthesis and aging are regulated by TOR pathway and many diseases including cancer have been reported to be the outcome of the deregulation of TOR pathway.

The immunosuppressant rapamycin is used in organ transplantation in order to prevent the organ rejection as well as the treatment of some cancer types. Rapamycin was also found to extend the lifespan in microorganisms and mice. Rapamycin acts in the cell by directly targeting the TOR complex. In addition to rapamycin, caffeine was also reported to affect the metabolism in a way similar to rapamycin and involved in lifespan extension. Like rapamycin, caffeine also targets the TOR complex but the exact mechanism of caffeine in cells is remains to be elucidated.

This study aims to elucidate the effects of rapamycin and caffeine on yeast metabolism. The effects of these chemicals on metabolic flux distribution were investigated using flux balance analysis. This study provides the identification of metabolic pathways altered in response to rapamycin and caffeine as well as the similarities and the differences between modes of action of these drug agents.

P24-7
SIMCHIP: prediction of transcription factor DNA binding landscape and position weight matrices evaluation

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A deep understanding of the dynamics of gene regulatory networks relies on the availability of models faithfully predicting the binding of transcription factors (TFs) to their DNA targets. Position Weight Matrices (PWM) describe nucleotide preference at each position of a TF binding site (TFBS) but, in addition to site presence, *in vivo* binding is modulated by several factors (TF binding competition, DNA accessibility, etc). In any case, an essential prerequisite is a good PWM that correctly establishes the interaction with potential TFBS. ChIP-chip/Seq experiments give a very useful TF binding picture but obtaining these data for all TFs, each tissue, developmental process, condition and developmental stage is unapproachable, even more in non model organisms. Optimization of PWMs allows the identification of gene regulatory networks in non-model species, and the possibility of manipulation of agronomically important traits.

Several algorithms are available to establish PWMs but they render significantly different results, so finding an evaluation method for the different models is prioritary. We propose a method, SIMCHIP, that simulates the binding for a given TF based on ChIP-chip/seq or SELEX data, and compares the output with *in vivo* data to evaluate PWM performance peak by peak. As a proof of concept, we have used this method on the plant-specific TF LEAFY and on the human TF STAT1.

SIMCHIP and other binding analysis tools based on PWMs are available at http://biodev.cea.fr/morpheus.

P24-8
Envelope morphology in nuclear compartmentalization and asymmetric segregation of nucleoplasmic factors during yeast closed mitosis

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During vegetative growth, *Saccharomyces cerevisiae* mother cells bud, producing smaller daughter cells. In this process, factors activating specific transcriptional programs are asymmetrically inherited. Thus, understanding how and when such asymmetry is established and maintained is critical, and we have now shown how compartmentalization of nucleoplasm and nuclear membranes depends on geometry and diffusion barriers, distinctively [1].

For this, we followed different steps of nuclear elongation and division, and correlated them with transcription factor localization. We then performed FLIP experiments for proteins in the nucleoplasm and nuclear membranes, revealing different compartmentalization patterns. Lastly, we considered nuclear geometry 'perturbations', by analyzing mutants yielding distinct internuclear bridge lengths and widths.

Simultaneously, we performed off-lattice spatial stochastic simulations with idealized nuclear geometries, based on fluorescence live-cell microscopy, and EM data in the literature. Diffusion rates were obtained by FCS and/or spatial simulations minimizing the error of the first moment as compared with experimental data. Parameter sweep simulations were then performed, to estimate optimal values of idealized diffusion barriers. Finally, spatial stochastic simulations of all FLIP scenarios in distinct nuclear compartments, were performed. Our simulations accurately match all FLIP experiments and controls, allowing us to quantify the separate roles of envelope morphology and barrier strength.

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P24-9
Competition between fatty-acid beta-oxidation enzymes makes the pathway vulnerable to substrate overload

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Lipid metabolism plays a key role in acquired and inborn metabolic diseases. Research is hampered, however, because the intermediate metabolites are difficult to measure. Computer simulations that mimic the dynamics of the involved metabolic pathways are an important tool in revealing underlying disease mechanisms.

Here, we present a dynamic computer model of fatty-acid beta-oxidation and apply it to understand the consequences of

fat overload. The model is built on known kinetic equations and measured kinetic parameters, which were based on literature data for rat-liver enzymes. The model predicts fluxes through the enzymes and intermediate metabolite concentrations and their dynamic response to perturbations.

To validate the model predictions, we have measured the oxygen consumption flux and the acylcarnitine concentrations in isolated rat-liver mitochondria upon addition of palmitoyl-CoA. The oxygen flux as well as the dynamics of the acylcarnitines showed correspondence between model and experiment.

Subsequently, we studied the effects of an overload of fatty acids such as often seen in obesity. This was done by increasing the palmitoyl-CoA concentration in the model. The model simulations showed that above a certain substrate concentration the pathway was overloaded, the flux dropped and metabolites accumulated. This was due to the competition between acyl-CoA dehydrogenases for substrates of different chain lengths, in combination with a finite coenzyme A concentration. The NAD+/NADH modulated the sensitivity to substrate overload, showing a tight interplay between beta-oxidation and respiration. In conclusion, computer simulations of fatty-acid beta-oxidation have yielded new insights and hypotheses about the mechanism of fatty-acid toxicity in metabolic diseases.

P24r-10
The human pathophenotype-causing genes network

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Diseases Networks are useful to study the molecular complexity of genetic diseases. Two main disease networks, 'the human diseases networks' (HDN, Goh et al. 2007) and 'the orphan disease networks' (ODN, Zhang et al. 2011), have been published to date among others. However, in these networks, each single node is a disease, characterized as a set of clinical features descriptions (pathophenotypes) represented as pathological entities. Most of these diseases were described using evidence-based medicine methods allowing physicians systematically to differentiate types and sub-types of diseases. Therefore, the representation of diseases as entities, without relationships to other phenotypically similar diseases, affects to network medicine methods. We hypothesize that the pathophenotypic relationships among diseases can help to find out interrelations in molecular events originated by mutations.

In this work, we built and analyzed the human pathophenome network to be compared to HDN and ODN. Unlike these previous networks, the pathophenome uses semantic similarities. The pathophenotypic similarities were calculated between pair of genes annotating phenotypic abnormalities in the 'Human Phenotype Ontology' and, subsequently, comparing gene phenotypic spaces. The resulting human pathophenome network contains 1706 genes (nodes) and 26 192 significant pathophenotypic similarities (edges). This network reveals a strong re-arrangement of the pathological relationships among genes and, moreover, they are measurable by phenotypic similarities. Many novel pathophenotypic interactions between genes have been uncovered. Our results indicate that pathophenotypes might contribute to discover pre-clinical stages and co-dependencies among disease-causing genes.

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P24-11
PhenUMA: a biomedical tool for the integration and visualization of phenotypic relationships among genes

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The integration of biomedical and biomolecular data is an efficient way to propose new hypotheses and intervention strategies for inherited diseases. Genetic disease databases (i.e. OMIM and Orphanet) give details about mutations and the molecular basis associated with pathological conditions. Different biomolecular interactomes can be used to integrate the underlying molecular and biochemical events including disease-causing genes. However, useful data integration tools require accurate methods to evaluate these relationships for large experimental datasets.

PhenUMA is a tool designed to integrate pathological relationships and functional interactions among genes. Different biomedical ontologies (HPO and GO) are used to calculate the phenotypic (HPO) and functional (GO) semantic similarities for large datasets of genes. Additionally, it is possible to retrieve different biochemical interactions based on physical interactions and metabolic flux correlations. This tool also allows for the calculation of phenotypic semantic similarities between a list of diseases, genes or clinical features and other genes or diseases.

PhenUMA is a novel and flexible application that makes use of a knowledge base to visualize different types of networks in different ways. The main objective is to provide friendly utilities for querying phenotypic profiles with the intention to enhance the integration of biomedical and biomolecular data with potential application in personalized medicine, especially in the case of orphan/rare diseases for which information availability for clinicians is essential.

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P24-12
A systems based approach to understand transcriptional differences of amylolytic saccharomyces cerevisiae strains developed for bioethanol production

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Limited oil reserves, increasing energy demand, fast growing population and concerns about climate changes have promoted global interest in renewable energy sources, especially biofuels. Among biofuels, bioethanol has been regarded as an important energy source. Bioethanol is produced mainly from starchy feedstocks by an energy-intensive, high-temperature cooking process followed by enzymatic hydrolysis of starch to fermentable sugars. Use of amylolytic yeasts for direct fermentation of starch in a simultaneous saccharification-fermentation process is an alternative to the conventional multi-stage process which is economically inviable in the long term. Hence, our research group is mainly focused on the development of non-cookingfermentation systems utilizing amylolytic yeast strains. In our previous studies, plasmid-bearing and integrant *S. cerevisiae* strains that can stably maintain and express multiple copies of DNA sequences encod-

ing *B. subtilis* α-amylase and *A. awamori* glucoamylase enzymes, were constructed. In addition to soluble starch, these recombinant amylolytic strains were also found to utilize raw starch substrates like wheat and corn starch. High starch utilization rate and stable amylolytic activity of these strains made them worthy of further investigation to elucidate their true potential in hydrolyzing starchy biomass resources as well as in other bioconversion processes. In this study, the constructed amylolytic strains were cultivated in fully controlled fermenters, their fermentation performances were compared and genome-wide transcriptional differences underlying strain performances were investigated by using a system based approach at transcriptional level.

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P24-13
Meta-analysis of clear cell renal cell carcinoma gene expression reveals the deregulated genes and their associated networks

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Background: Microarray gene expression profiling has been used in the past by various groups to identify novel markers of clear cell renal cell carcinoma (ccRCC).
Aim: We hypothesized that a meta-analysis of publicly available gene expression datasets of ccRCC may identify a list of the common deregulated genes (DEGs), which should have higher potential to be used as novel tumor markers.
Methods: In the Oncomine platform, genes deregulated in ccRCC relative to the corresponding normal tissue were filtered by a corrected Q value cut-off and concept filters. The identified genes that were common among five different microarray datasets were chosen as the candidate markers and their performance to discriminate between cancer and normal tissue was measured by ROC test. Their networks were analyzed by Ingenuity Pathway Analysis. Further Gene Ontology enrichment, KEGG pathways and Transcription Factor Target analyses were performed for the most common DEGs.
Results: We concluded in 93 commonly up-regulated and 76 commonly down-regulated genes in ccRCC versus the normal tissue. The top 10 up-regulated molecules were NDUFA4L2, PLIN2, NNMT, ENO2, AHNAK2, NETO2, CA9, VWF, COL23A1 and EHD2; whereas the top 10 down-regulated molecules were NPHS2, CALB1, RALYL, KCNJ1, KNG1, SERPINA5, CLDN8, SLC12A3, CA10 and ATP6V0A4. The top canonical pathways included the antigen presentation pathway, the inositol metabolism and the pentose phosphate pathway among others. The common DEGs participate in biological functions such as cancer, inflammatory response, renal and urological disease, reproductive system disease and respiratory disease. The top molecular and cellular functions were cell-to-cell signaling and interaction, cellular function and maintenance, molecular transport, cellular growth and proliferation and carbohydrate metabolism.
Conclusions: This meta-analysis highlights the genes most likely implicated in ccRCC. The genes that exhibit the best discriminatory performance between ccRCC and normal tissue are suggested as predictive markers for the disease.

P24-14
Central metabolism adaptations for ectoines synthesis in *Chromohalobacter salexigens*

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The halophilic bacterium *Chromohalobacter salexigens* is able to grow up to 3 M NaCl in minimal medium due to the internal accumulation of ectoines as compatible solutes. The synthesis of ectoines in *C. salexigens* is an osmoregulated process that draws intermediaries from the TCA cycle. Consequently, its central metabolism has to adapt to support this biosynthetic route.

In the present study, *C. salexigens* was grown in batch using minimal medium with 0.75 and 2.5 M NaCl, and [1-^{13}C]-, [2-^{13}C]-, [6-^{13}C]- and [U-$^{13}C_6$]-glucose as the carbon source. The utilization of glucose and ammonia and the organic acids production were profiled during growth. The consumption rates reflected a more efficient metabolism at high salinity, whereas at low salinity, unbalanced growth led to metabolic overflow, and organic acids were found in the supernatant (gluconate, pyruvate and acetate), along with a lower biomass yield.

Tracing of isotopic labels allowed us to determine the main central metabolism pathways involved in ectoines biosynthesis from glucose, as well as the estimation of key metabolic flux ratios. Such results, supported by a bioinformatic analysis of *C. salexigens* metabolism and genes, helped us to conclude that this bacterium uses preferably the Entner-Doudoroff route for glucose catabolization while metabolic flux ratios point to a high anaplerotic activity for replenishing the TCA cycle with the intermediaries withdrawn for ectoines biosynthesis, also supported by *in vitro* assessment of high pyruvate carboxylase (Pc) activity. Both Pc level and anaplerotic fluxes were high at any salinity. Altogether, these results suggest a certain metabolic rigidity in *C. salexigens*, indicating its specialization in order to support high biosynthetic fluxes and partly explaining why metabolic yields are so highly affected by salinity.

P24-15
Possible impact of invasive algae *Gonyostomum* on the lakes microflora in Lithuania

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For the primary analysis was chosen the biological system of the invasive algae *Gonyostomum* and their microorganisms. Special attention was devoted to concentrated algae fraction, because in this case bacterias that are not related with them concentrate very hardly. After five lakes study, where mostly spread algae are *Gonyostomum*, was determined that in their fractions only two species were yeasts and dominates bacterias, where about 30% have the adhesive features. It may be related with the processes of symbiosis. Fractions of control lakes where was not found microflora of *Gonyostomum* had different species of microorganisms and differed visually. Therefore it can be assumed, that these algae form self-specific microflora. To evaluate their biocidal activity *S. cerevisiae* K1, K2, K28 killer systems and sensitive strains were used. In concentrated algae fractions six clones

with different biocidal activity were found. It was identified, that the most active clone for the biocidal features is N2. It is about four times more active than *S. cerevisiae* K1 killer toxin. It was determined, that this clone was lethal for *Penicillium chrysogenum*, *P. cyclopium*, *P. verrucosum*, *Aspergillus flavus*, *A. terreus*, *Fusarium poae*. The zones of lysis were 15–30 mm. The other clone S1 acts on *Aspergillus terreus*, *A. flavus*, *Penicillium chrysogenum*, *Fusarium poae*. The zones of lysis were 6–13 mm. Other clones have smaller biocidal activity. Hypothetically these bacterias may affect the spread of *Gonyostomum*. After comprehensive investigation of these bacterias and genes, encoding toxins may be used producing new biopreparations, medicaments (new antibiotics), modified organisms. The research shows, that the biocenosis of lakes when dominates algae *Gonyostomum* is changing microbiologically.

P24-16
System analysis of the role of Acetil CoA Synthetase acetylation in a *E. coli* transient diauxic system

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Diauxia enables *E. coli* to shift from glucose to acetate as carbon source. Acetil CoA Synthetase (ACS) is a crucial enzyme in glucose/acetate diauxia, scavenging acetate from media when glucose is absent. Based on experimental data obtained from aerobic glucose batch cultures of *E. coli*, we have devised a model on GMA power-law formalism aiming to capture the dynamic behavior of ACS system during transition from glucose to acetate in batch cultures. The model is aimed to unravel and quantify the importance of the regulating mechanisms involved in this transition. These mechanisms range from metabolic (cAMP synthesis) to transcriptional and post-translational level. Special attention is paid to the role of ACS acetylation/deacetylation genes, its regulation by cAMP-CRP (Castaño-Cerezo et al., 2011) and its implications on acetate metabolism regulation.

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P24m-17
Mathematical model of the development of Atopic dermatitis

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Atopic dermatitis (AD) is one of the most common skin diseases affecting the paediatric population worldwide. Despite the high impact of AD on public health, the mechanisms leading to this disease remain largely unknown due to the complexity of the underlying cellular processes. AD is characterized by a physical disruption of the skin barrier and a hypersensitive immunological response to environmental stimuli. The proteolytic enzyme KLK plays a pivotal role in these processes, as it degrades intercellular junctions, reduces the lipid content of the skin barrier and induces an immune reaction by activating the receptor PAR2. Accordingly, the development of AD can be associated with a disruption in the regulatory mechanisms of the KLK activity.

We propose the first mathematical model of the complex network of pH-dependent protein interactions that regulate KLK activity. Bifurcation analysis of our model described by ordinary differential equations shows bistability that is able to capture key features of the inflammatory response found in AD patients, such as hypersensitivity to allergens and persistence of inflammation. The model predictions are also consistent with microarray data.

The deregulation of the proteolytic KLK activity also affects epidermal cohesion, leading to an impaired skin barrier that becomes more sensitive to allergens. This interplay between KLK activity and the physical integrity of the skin barrier is modelled at different time scales through an extended model that captures the dynamic changes in the inflammatory response corresponding to the development of AD.

This work contributes to the understanding of how skin inflammation develops in AD and offers clinically relevant predictions, showcasing the advantages of using a systems biology approach to understand the mechanisms underlying complex diseases such as AD.

P24-18
Deciphering keratinocyte-fibroblast communication in the human skin: who is talking?

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Cell-cell communication between epithelial human keratinocytes (NHK) and fibroblasts (HDF) is essential for skin homeostasis, regulation and regeneration. This double paracrine crosstalk is mediated through various cytokines, whose particular composition defines cell specific responses such as cell proliferation, migration, differentiation or death. While many of these secreted factors have been studied individually, their synergistic action is largely unknown. Here, we study the time-ordered dynamics of double paracrine cell-cell communication in the skin to unravel the mutually dependent regulation of keratinocytes and fibroblasts. We decomposed the cell communication between HDF and NHKs into unidirectional and consecutive paracrine stimulation steps with the help of conditioned medium. We found, and confirmed in co-culture, that fibroblasts respond to conditioned NHK medium with a well-defined change in the transcriptome and secretome mimicking an inflammatory response via NfκB pathway. Keratinocytes, on the other hand, do not react significantly to supernatant derived from only fibroblast monoculture, yet respond to the double paracrine stimulation and co-culture. These results suggest that keratinocytes actively request factors from the mesenchyme (HDF) to establish their micro-environment via inflammation processes, resulting in an increased migration of keratinocytes that only occur after crosstalk with fibroblasts. To decipher the sequence of events leading to the coordinated secretion of HDF-derived cytokines, we established both a mixed-effect model, regarding the paracrine stimulation and response as multiple-input multiple-output system and a gene regulatory model of the transcriptome response in HDF to NHK supernatant. The data analysis and modeling showed a distinct

combination of mainly interleukins and several late regulated cytokines as necessary and sufficient players through which cell-cell communication is established and which allowed for a non-autonomous control of keratinocyte behavior.

P24-19
Distributed biological computation with multicellular engineered networks

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Ongoing efforts within synthetic and systems biology have been directed towards the building of artificial computational devices using engineered biological units as basic building blocks. Such efforts, inspired in the standard design of electronic circuits, are limited by the difficulties arising from wiring the basic computational units (logic gates) through the appropriate connections, each one to be implemented by a different molecule. Here, we show that there is a logically different form of implementing complex Boolean logic computations that reduces wiring constraints thanks to a redundant distribution of the desired output among engineered cells. A practical implementation is presented using a library of engineered yeast cells, which can be combined in multiple ways. Each construct defines a logic function and combining cells and their connections allow building more complex synthetic devices. As a proof of principle, we have implemented many logic functions by using just a few engineered cells. Of note, small modifications and combination of those cells allowed for implementing more complex circuits such as a multiplexer or a 1-bit adder with carry, showing the great potential for re-utilization of small parts of the circuit. Our results support the approach of using cellular consortia as an efficient way of engineering complex tasks not easily solvable using single-cell implementations.

P24-20
Simulating diffusion in crowded environments with multifractional Brownian motion

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Diffusion of molecules within cells has many times been measured and characterized as highly anomalous. Even though the underlying mechanisms are not entirely understood, excluded volume effects inside the crowded cell have already been identified as one cause for subdiffusion. A side from experimental studies, computer simulations have been used to deepen our understanding of anomalous diffusion. In the past, the focus has been largely on continuous time random walks (CTRWs), but recent work has suggested that fractional Brownian motion (FBM) may be a better descriptor of diffusion in crowded environments. FBM is driven by a Gaussian process with zero-mean and a covariance function that depends on the so-called Hurst exponent H. A natural generalization of FBM is achieved by replacing H by a time-dependent Hölder function H(t) leading to multifractional Brownian motion (MFBM).

Here, we present results from a recent study using FBM and MFBM to simulate diffusion of a tracked particle in the presence of crowding molecules and physical obstacles. Particle tracking data, mimicking experimental data, was first generated with an off-lattice particle simulator. We then attempted to obtain computationally significantly less expensive (M) FBM paths that match the statistical properties given by mean-square displacement (MSD) and time averaged MSD of our sample data. While diffusion around immovable obstacles can be reasonably characterized by a single Hurst exponent, diffusion in the presence of crowding molecules seems to exhibit multifractional properties in form of a different short and long-time behaviour.

P24-21
Effect of osmotic stress on the regulatory network of *E. coli*

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The addition of salt is one of the most widely used means of preservation of food, and has been for centuries. Food-borne pathogens subjected to osmotic stress have first to re-establish their turgor by importing ions such as potassium into the cell and then replace, at least partly, these ions with osmoprotectants to resume growth. However, the more salt is added to the medium, the slower the growth and it is not well understood what limits the cells ability to grow. In order to determine the strategy of the cell facing osmotic stress, we analyse the regulatory network of *E. coli* in exponential phase as a function of NaCl concentration and osmoprotectants commonly found in food (glycine betaine, choline and proline) in a minimal medium. We integrate micro-array measurements into a regulatory network reconstructed from data from the literature and follow its evolution as a function of the environmental factors. The results suggest that, overall, the cell follows a cost minimisation strategy and that some pathways are specific to particular osmoprotectant.

P24-22
Identification of a functional human gluconokinase through systematic expansion of a human metabolic network

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Metabolic network reconstructions define metabolic information within a target organism and can therefore be used to contextualise incomplete metabolic information. Here, we used a computational approach to identify human metabolites whose metabolism is incomplete on the basis of their detection in human bio-fluids but exclusion from the human metabolic network reconstruction RECON 1. Candidate solutions, composed of metabolic reactions capable of explaining the fate of these compounds in humans, were then identified computationally from a global biological reaction database. Solutions were characterised with respect to how metabolites were incorporated into RECON 1 and their biological relevance. Through detailed case studies, we show that biologically plausible hypotheses regarding the metabolism of these compounds can be proposed in a semi-automated manner in an approach that is similar to de novo network reconstruction. As proof of principle we experimentally confirmed one proposed hypotheses that involved the functional validation of a previously uncharacterised human gluconokinase. Metabolic

reconstructions are inherently incomplete. The results demonstrate how metabolic network models can be used to define and incorporate incomplete biological information resulting in models with greater biological scope. Furthermore how the definition of incomplete metabolic knowledge can fuel successful experimental research through the prioritisation of biological unknowns.

P24-23
Applied systems biology – vanillin production in *Saccharomyces cerevisiae*

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Vanillin is the most important aroma compound based on market value, and natural vanillin is extracted from the cured seed pods of the Vanilla orchid. Most of the world's vanillin, however, is obtained by chemical synthesis from petrochemicals or wood pulp lignins. As an alternative, de novo biosynthesis of vanillin in baker's yeast *Saccharomyces cerevisiae* was recently demonstrated by successfully introducing the metabolic pathway for vanillin production in yeast. Nevertheless, the amount of vanillin produced in this *S. cerevisiae* strain is insufficient for commercial production and improvements need to be done. We have introduced the genes necessary for vanillin production in an identical manner in two different yeast strains S288c and CEN.PK,where comprehensive – omics datasets are available, hence, allowing vanillin production in the two strain backgrounds to be evaluated and compared in a systems biology setting.

P24-24
Kinase control of lipid homeostasis in mammalian cells

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Lipidomics is an emerging field, focused on the large-scale study of the lipids arrangement in a specific biological system. There is a great heterogeneity of lipid structures in biological systems. This diversity is present at every levels of organism organization: between cell types, organelles, and between the two leaflets of the lipid bilayer. In Mammalian cells, homeostasis of membrane lipids is essential in order to conjointly maintain the structure of the system, responses to cell environment, trafficking and signaling events. Disruption of this balance leads to physiological disorders, such as the Niemann-Pick disease, neural and cardiovascular diseases, cancers, etc. If more and more genes are found to be involved in homeostatic mechanisms, no systematic view of membrane lipid regulation is available, yet. My work is centered on this issue. I research genes from the Human kinome necessary in the control of membrane lipids homeostasis. Combining techniques of gene silencing (siRNA), lipid extraction, Mass Spectrometry (TSQ) and Bioinformatics, I quantify the lipid composition of HeLa cell membranes in the absence of targeted genes. Then, the computation of lipid profiles, allows the clustering of lipid variations according to protein knockdown, leading to the elaboration of new hypotheses about general mechanisms of membrane lipids homeostasis.

P24-25
Investigation of alterations in *S. cerevisiae* gene expression in response to chronological aging

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Cellular mechanisms leading to aging and therefore increasing susceptibility to age-related diseases are a central topic of research since aging is the ultimate, yet not understood mechanism of the fate of a cell. Studies with model organisms have been conducted to ellucidate these mechanisms, and chronological aging of yeast has been extensively used as a model for oxidative stress and aging of postmitotic tissues in higher eukaryotes. In this study, a comparative analysis of the transcriptome profiles of chronologically 'young' and 'old' yeast cells was conducted. Wild-type (BY4742) cells were grown in SDC to postdiauxic-shift phase in 2L fermenters with 1L working volume, at 30°C and 400 rpm, with constant air flow at a rate of 1 vvm. Samples for transcriptome analyses as well as life span assays were collected at the 4th and 7th days for young and old cells respectively. The comparative analysis of these expression profiles offers a global perspective which might be helpful to discover the major regulatory pathways and targets affecting the chronological life span of yeast.

P24-26
Estimation of metabolite concentration ranges in human cells inflicted with inborn errors of metabolism

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The second law of thermodynamics implies that net flux through a biochemical reaction is always in the direction associated with a negative transformed Gibbs energy. Transformed reaction Gibbs energy is a function of the concentrations of reactants and products. In a metabolic network, the concentrations of metabolites need to be such that the second law is upheld for all reactions in the network. If steady-state flux directions and transformed Gibbs energies are known for reactions in a metabolic network, they can be entered as constraints in a linear programming problem to estimate bounds on the concentrations of metabolites in the network.

We have previously estimated bounds on transformed Gibbs energies for reactions in the human metabolic reconstruction Recon 1. These bounds were used to constrain metabolite concentration ranges in healthy human cells. Concentrations were further constrained by steady-state flux directions for a small set of reactions that had consistent directionality in 280 flux distributions predicted by flux balance analysis. Even with such loose constraints we were able to estimate concentration ranges for 20 metabolites. In the next phase of this study we will compare estimates of metabolite concentration ranges in healthy human cells and cells where genes linked to known inborn errors of metabolism (IEMs) have been knocked out. Predicted concentration differences will be validated against data from the IEM screening

program for newborns at Landspitali-University Hospital in Iceland. Ultimately, we hope to predict new markers for IEMs that are currently not easily diagnosed.

P24r-27
Simple kinetic constraints in a compartmentalized protometabolic system: a semi-empirical approach

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A key question in the origins of cellular life is how difficult was for protometabolic reaction networks to start developing within compartments, long before becoming complex (genetically-instructed and enzymatically-regulated) coupled biochemical cycles. This work is conceived as a systems biology approach to find out to what extent realistic prebiotic compartments, like fatty acid vesicles, would constrain the chemical network dynamics that could sustain a minimal form of metabolism. We combine both experimental and simulation results to establish the conditions under which a simple reaction network capable to produce its own catalysts would overcome the potential problem of 'self-suffocation' that arises from the hindered accessibility of nutrients to its internal reaction domain. The relationship between the permeability of the membrane, the lifetime of the key catalysts and their efficiency (reaction rate enhancement) turns out to be critical, as derived from our analysis. Through this concrete and illustrative example we would like to foster a necessary discussion about this topic within a more general, evolutionary perspective.

P24r-28
Whole-genome transcriptomic analysis in *Arabidopsis thaliana* unveils interactions between carbon metabolism and the photoperiodic pathway

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Starch is the major carbon reserve in higher plants. On the one hand, the enzyme granule-bound starch synthase (*GBSSI*) plays a key role in starch synthesis. On the other hand, the gene *CONSTANS (CO)* is central to the photoperiodic pathway [1]. In order to analise the interactions between carbon metabolism and the photoperiodic pathway our group is performing a whole-genome transcriptomic analysis in different photoperiodic pathway and carbon metabolism mutants that include *GBSSI* and *CO* mutants.

We have constructed a gene co-expression network based on the expression profiles estimated from microarray experiments. Using the R statistical programming language we determined the differentially expressed genes and the significant interactions among them. These were imported into Cytoscape [2] in order to construct our network. A clustering analysis based on Pearson correlation and a functional enrichment based on Gene Ontology annotations helped us identify four different clusters in our co-expression network: a central cluster constituted by carbon metabolism genes such as *APS1*, *TPS8*, and *GPD*; a peripheral cluster with genes associated to the photoperiodic pathway such as *CO* and *FT*; another cluster with genes involved in the response to stimuli such as *GBF3*, PHYC and COR15 and finally a cluster formed by genes involved in the transport of organic substances such as *ERD6*, *UTR2* and *GBSSI*. These results suggest that genes such as *GBSSI* are not solely involved in carbon metabolism but rather they serve as an interface between flowering regulation, transport and carbon mobilisation.

P24-29
Systematic analysis of specificity determinants of human protein kinases

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Human protein kinases are involved in essentially every cellular process. Despite years of research, the problem of kinases substrate specificity is far from being completely understood, probably due to the specificity is determined by several factors in addition to the recognition of motifs in the sequence of the substrate. During the last years phosphoproteomics methods had increased our knowledge on phosphorylation sites, providing us with data that can be readily used in systematic analysis of kinases specificity determinants. Here we propose an ongoing analysis of the substrate specificity of human kinases that integrates information from kinases catalytic domain sequences, structures and kinases phosphorylation profiles. From available online resources, we have compiled data on experimentally determined phosphorylation sites for 325 human kinases comprising 93 kinases families. We have analyzed the specificity of these kinases and kinases families using PSSM models derived from their corresponding phosphorylation sites. Our results suggest that the statistical significance of our PSSM models, computed in terms of the p-value of the information content (IC), does not depends on the number of phosphorylation sites from which the PSSMs are generated; and that the percent recall and IC of our PSSM depends on the number of phosphorylation sites. Our results also suggest that the scarce information on the phosphorylation profile of a certain kinase (i.e. experimentaly determined phosphorylation sites) might be causative of artificially high values of recall and IC.

P24-30
OralCard: a bioinformatic tool dedicated to the oral cavity system

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The elucidation of the molecular mechanisms that participate on biological systems and the generation of meaningful and applicable biological information depends on the experimental/technical skills to identify the molecules involved in a particular process and which are present/absent/altered in a defined situation and the existence of tools to facilitate the organization and integration of the information generated which is mostly dispersed. The molecular mechanisms occurring in the oral cavity depend on the dynamic balance and functional integration of two molecular

universes, one corresponding to the host functions and the other resulting from the microbiome present. The understanding of the role of proteins, both human and microbial, in the molecular processes within the oral cavity will be a fundamental contribution to the understanding of oral biology and a translational contribution to the design of preventive and therapeutic strategies with implication on oral health. In addition, since the oral cavity collects proteins from different systemic sources which are reflected in whole saliva composition, this fluid has been considered as an interesting target for systemic disorders biomarker discovery. In this work we present OralCard, an online bioinformatic tool, that comprises proteomic results and more targeted studies, reflecting the Oral Molecular Ecosystem (OralPhysiOme) by merging the experimental information available from the oral proteome both of human (OralOme) and microbial origin (MicroOralOme). OralCard has three main objectives. It is a repository of all the information available on the proteins of human and microbial origin present in the oral cavity. The information of the proteins will be permanently updated. Another objective of OralCard is to allow easy and direct access to the information on proteins of the oral cavity both to a specialized and general audience, by permitting searches through protein, disease and organism. As a last objective, OralCard aims at storing and displaying information on whether a certain protein of the oral cavity (regardless of its human or microbial origin) has been identified or proposed as a biomarker of oral or systemic conditions. Oralcard is available at http://bioinformatics.ua.pt/oralcard.

P24-31
Oral microbial proteome: clues of OralCard

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Proteomic analyses of saliva have been extensively published but only a few studies report microbial proteins in their results. The goal of this poster is to present the microbial proteins identified in proteomic studies of saliva and other oral samples. Using the database OralOme and the web interface OralCard, microbial proteins experimentally identified in saliva are explored and classified regarding their ontological classifications.

Only five proteomic studies using oral tissue samples, have identified microbial proteins. One of these studies reports the majority of microbial proteins (1008 in 1211). The proteins identified correspond to 272 microbial species and strains representing 96 bacterial genera two viruses and one yeast.

When classified according to their ontological annotations, we observe that about half of the proteins (50.1%) are intracellular. Thirteen point seven proteins are included in membranes or extracellular space, having the potential to interact with host derived molecules. The remainder 37.1% are classified with ontologies which are not specific, such as cell component.

When the biological process is considered, 24.27% of the proteins have an unknown biological process annotation. There are in total 34 ontological annotations for biological process most of which relate specifically to the bacterial cell. Five proteins are annotated as being involved in cell communication and three are related to symbiosis, encompassing mutualism through parasitism, making them interesting for the study of host-microbial interaction. A similar scenario arises when molecular function is considered; the most abundant functions (15%) are catalytic activity and binding, which are generic and not very informative. Of the other 27 ontological annotations, transporter activity, peptidase activity, protein binding, antioxidant activity, carbohydrate binding, receptor activity, receptor binding, calcium ion binding and lipid binding emerge as the potentially most relevant when host-microbial interaction is of interest.

These results allow the following conclusions: (i) the microbial species known to exist in the oral cavity are underrepresented in proteomic studies of oral samples; (ii) microbial proteins are not well annotated; and (iii) there is evidence of the presence of proteins that might be important in the host-microbial interaction such as those annotated as being involved in symbiosis. More experimental data focused on the microbial proteome of the oral cavity is needed to fully understand this ecosystems biology.

P25 – Theoretical Molecular Biology

P25-1
Detection and characterization of genes related to avian pathogenic *Escherichia coli* APEC in wild birds by PCR (Polymerase Chain Reaction)

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Escherichia coli are present in large numbers in the intestinal tract of most animals, some specific strains are associated with diseases. For birds, these strains are called APEC. The detection of of *E. coli* strains carrying virulence genes associated with APEC in wild birds may present a risk to animal health protection, because wild birds hauling these genes could serve as reservoirs of strains pathogenic to birds. The need to gain a better understanding of the APEC related genes in order to classify the APEC pathotype is the motivating factor behind this study, which aims to determine the frequency of these genes, while characterizing the isolates genes related to APEC. One hundred and six cloacal samples and 106 oropharynx samples were collected from wild birds treated at the clinic of Wildlife Veterinary Hospital of UNESP-FCAV, totaling 212 samples. The samples were first enriched and subjected to screening by PCR to determine the genes *iroN, ompT, iss, iutA, cvaC* and *hlyF*. Of all the cultures that showed the presence of any of the researched genes, there were ten isolated colonies that were again analyzed by PCR to find isolates containing the genes once the new PCR isolates were obtained and identified as APEC-related. From these isolates, a new PCR was performed to find *sitA, irp2, fyuA, tsh*. Of the 212 samples, there were 20 isolates obtained (11 from cloaca and nine from oropharynx), 12 were positive only to sitA, three *to irp2, fyuA, sitA* and *tsh*, two to *irp2, fyuA* and *sitA*, one to *irp2* and *fyuA* and one to *irp2* and *sitA*. The results showed that samples from wild birds show the presence of *E. coli* containing the virulence genes associated with APEC. This shows that wild birds can be reservoirs of potentially pathogenic bacteria for commercial poultry.

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P25-2
Theoretical investigations on o-substitue derivatives of ısoflavans: SAR and QSAR Study

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Theoretical analysis for the structural and conformational properties of the o-substitute derivatives of isoflavans; Arvensan (A), Isosativan (Is), Isovestitol (Iv) and Sativan (S); is investigated using DFT method in combination with the 6-31G basis set. Isoflavans, a group of flavonoids, have many beneficial pharmacological effects on human health. Pharmaceutical and environmental properties have been used for structure activity relationships (SAR) and quantitative structure activity relationships (QSAR) investigations. All theoretical calculations were performed with the HyperChem 7.5 and Gaussian 03 program package. To calculation results, we can interpret that the molecules have hydrophilic (water loving) or hydrophobic (water fearing) properties. We found that the molecules are all water-soluble (polar) molecules because they have negative logP value. LogP values for A, Is, Iv, and S molecules are -1.49, -1.49, -1.52 and -1.49, respectively. Moreover, for the pharmacological application, logP value is a useful tool because of the extrapolating distribution of drugs within the body. Another parameter for determination of molecules solubility is hydration energy value. Hydration energy values for A,Is,Iv and S are -10.66, -8.08, -16.92 and -11.98 kcal/mol, respectively. Iv is more soluble than other molecules in the water because it has the largest hydration energy value. For the molecules carried out, polarizability values are close to each other. Polarizability (A^3) values for A,Is,Iv and S are 31.04, 31.04, 29.21 and 31.04, respectively. Iv molecule has the smallest refractivity and polarizability value in this study. These results can be used in designing new drugs in a way of economic in the future.

P25-3
Understanding protein dynamics with coarse-grained models: from structures to disease

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The epidermal growth factor receptor (EGFR) family is the prototype of protein tyrosine kinase receptors, which are key regulators of cell growth. In the presence of ligands, the extracellular domain undergoes a dramatic conformational change from a closed, self-inhibited monomer to an open form, which dimerizes triggering cytoplasmic kinase activation. In the brain tumor *glioblastoma multiforme*, clusters of point missense mutations have been identified in this extracellular domain (1), associated with an increased activity, and presumably, a missregulated open conformation. Here we explore the dynamics of the ectodomain using an Elastic Network Model (ENM) (2), which suggests that the entire structure behaves like a macromolecular spring with an intrinsic opening controlled by strategic contacts. Perturbation schemes from ENM (3) detected critical regions for the conformational dynamics, located at highly conserved interdomain contact regions, involving mutations in both surfaces. Extended Molecular Dynamics (MD) simulations of both wild-type receptor and activator mutants, displayed spontaneous domain rearrangement even in the ns timescale towards different functional states. Overall, the results show that although protein motion is mainly encoded in the global structure, minimal changes in critical points can have a large dynamical impact. Furthermore, the surprising correlation found between the conformational change, MD simulations and normal modes, provide a strong evidence that multidomain proteins may explore a highly harmonic energy landscape.

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P25-4
NMR chemical shifts and protein dynamics

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Chemical Shifts are one of the most basic observable of NMR. In my current research I am trying to exploit their content through the use of statistical models and Molecular Dynamics simulations. d2D is a tool that interprets the chemical shifts in terms of quantitative populations of secondary structures. This tool can be used to easily characterise the low structural content of Intrinsic Disordered Proteins for example as a function of mutations or different environments. CamShift is a tool that predicts the backbone chemical shifts from the structure of a protein, in this way enabling the use of Chemical Shifts as a structural restraint in MD simulations. A replica-averaging approach has been used to characterise the structural fluctuations of the native state of RnaseA and is now been used to characterise intermediate and disordered states of different protein systems. A series of preliminary results are shown comparing the two approaches and cross validating them.

P25-5
B-DNA polymorphisms at the base pair step level

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DNA duplex is a flexible and polymorphic molecule but the available data of the ten unique representative base pair steps that nowadays exist is still scarce from experimental sources. There is even a more complex scenario if we consider the tetramer level where many of the 136 possibilities are not present in the current databases (1–3). Certain base pair steps show bimodal distributions instead of the usual unimodal Gaussian-like shape. The CG step, the most abundant in the available databases, shows this abnormal behaviour in the twist helical parameter, which describes one of the relative rotations between the base pairs of a base pair step. Analysis of the CG steps contained in the main structural databases shows bimodal distributions depending on their nearest neighbours (4,5). The bimodality present in some of the base pair steps, and therefore their separation from the Hooke's law, would have non-negligible consequences in DNA deformability and therefore, in the study of the genomic DNA and its elastic properties.

Interestingly, MD simulations allow the possibility to greatly increase the single representation of every step with different flanking bases. This task has been performed in a methodical fashion by the Ascona B-DNA Consortium in the last years and the results have demonstrated the suitability of this method to correctly represent B-DNA polymorphisms (4,5). Here we will present the main differences at the base pair step level between theoretical and experimental databases as well as a more detailed study on the CG step and its bimodal behavior. The ionic interactions with DNA together with relative motions of specific dihedral angles in the backbone seem to be involved in the coexistence of two states, low and high twist, of this particular base pair step.

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P25-6
Addressing macromolecular flexibility

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The prediction of molecular collective intrinsic motions is valuable for both understanding the functional conformational changes and introducing flexibility into the molecular modeling applications, especially for large systems. We recently developed a new normal mode analysis framework in internal coordinates, named iMOD, (http://chaconlab.org/imod/index.html) to approximate protein and nucleic acid flexibility. We will illustrate the sampling power of this approach in diverse scenarios including conformational exploration and flexible docking/fitting. We will also present some complementary advances on loop modeling using geometric algebra.

P25r-7
Physical characterization of the functionality of DNA from molecular dynamics simulation

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Genomic studies provide the 1D information encoded in DNA, but we cannot ignore that in eukaryotic organisms, DNA is packaged into chromatin structure, which folds DNA to a global compaction of at least 104. Genome homeostatic histone concentration ensures that most of DNA is wrapped into nucleosomes (~75–90%), constituting structural units of 147 base pairs (bp) long, and short linkers (around 20 bp long in yeast).

Therefore, DNA compaction has clear functional implications, since it modulates the accessibility of genomic regions to regulatory proteins. Indeed, a close relationship was established between nucleosome positioning and important regulatory signals, such as proximal promoters or splicing sites. The underlying DNA sequence has long been considered as an important contributor to nucleosome assembly, but the crystal structures of nucleosome core particles failed to detect any direct readout mechanisms of histone octamers for the DNA sequence, which led to postulate that histone-DNA interactions are not the major determinant of nucleosome positions *in vivo*. Nevertheless, the extent to which nucleosome positioning *in vivo* is dictated by the DNA sequence is a continuing debate far from being solved. Several theoretical studies carried on by Prof. Orozco's group and others have indeed provided indirect support to the connection between DNA physical properties and chromatin organization. Unfortunately, early used descriptors are rather poor, based on force-field that might be ameliorated in important aspects such as: reproduction of bimodality in some helical parameters, incorporating the impact of epigenetic modifications, capturing the importance of non-neighboring effects and the physical effect of unspecific charge neutralization.

P25-8
Protein disorder in plants: a view from the chloroplast

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The intrinsically unstructured state of some proteins, observed in all living organisms, is essential for basic cellular functions. In this field the available information from plants is limited but it has been reached a point where these proteins can be comprehensively classified on the basis of disorder, function and evolution. Our analysis of plant genomes confirms that nuclear-encoded proteins follow the same trend than other multi-cellular eukaryotes; however, chloroplast- and mitochondria- encoded proteins conserve the patterns of Archaea and bacteria, in agreement with their phylogenetic origin. Based on current knowledge about gene transference from the chloroplast to the nucleus, we report a strong correlation between the rate of disorder of transferred and nuclear-encoded proteins, even for polypeptides that play functional roles back in the chloroplast. These observations suggest that the evolutionary dynamics of the plant nucleus adds disordered segments to genes alike, regardless of their origin, with the notable exception of proteins currently encoded in both genomes. We further investigate this trend by reviewing the set of chloroplast ribosomal proteins, finding that the ribosomal large subunit, assembled from a majority of nuclear-encoded proteins, is clearly more unstructured than the small one, which integrates mostly plastid-encoded proteins.

P25-9
Prevalence and characterization of shigatoxigenic (STEC) and enteropathogenic (EPEC) Escherichia coli strains from fishes for human consumption isolated by Polymerase Chain Reaction (PCR)

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The expansion of aquaculture, the increased consumption of fish meat along with the precision of molecular biology techniques, make research related to virulence genes in animal products a global need and trend. Although *Escherichia coli* is not a natural organism of the intestinal tract of fishes, it is known that the microbiota of these animals is directly related to the microbiological quality of water that they live. Shigatoxigenic (STEC) and enteropathogenic (EPEC) *Escherichia coli* are important pathogens in public health due to transmission of enteric diseases to humans and the possible emergence of multiresistant isolates. Based on this, this work aims to detect STEC and EPEC strains by PCR, to characterize virulence genes of isolates from the intestinal contents and fish muscle, and to evaluate the susceptibility of these isolates against several antimicrobial agents. Of the 200 fish samples analyzed, one (0.5%) were positive STEC because the *stx*2 gene was present. Besides, this strain was positive for the virulence genes *ehx*A, $lpfA_{O113}$ and *saa*. This strain showed resistance profile in relation to antimicrobials: ampicillin, cephalothin, streptomycin, gentamicin, ciprofloxacin, chloramphenicol and tetracycline. The low frequency found and the multiresistance of the strain isolated shows that antimicrobials are used indiscriminately in fish farming, further aggravating the results found. Furthermore, the fishes can transmit STEC, affecting the humans health and conveying multiresistant pathogens.

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P25-10
Molecular identification of virulence genes in the region Lee and non Lee of enteropathogenic Escherichia coli (EPEC) isolated from humans and animals

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Diarrheagenic *Escherichia coli* strains are major pathogens associated with enteric disease worldwide. Escherichia coli enterophatogenic (EPEC) is a category *E. coli* of interest in public health, since it is responsible for causing diarrhea, especially in children in developing countries. The genes necessary for the establishment of A/E lesions are located on the pathogenicity island designated as LEE. Some proteins, secreted by the bacteria, are part of a discharge apparatus, while others are effector proteins that interfere with a variety of cellular processes. Various effector proteins encoded outside region LEE have been described in EPEC. The role of some of these effectors is not well defined, but we know that some of these are associated with diarrhea, as nleE. The aimed of this study was determined the presence of genes related of the region LEE and non-LEE of EPEC isolates. We tested 60 strains of EPEC (isolated from humans, buffaloes, pigs and sheep in Brazil) and the presence of genes bfp, nleA, nleE, espB (EDL933) and espD (EDL933). The samples were first subjected to screening by PCR for the detection of the eae gene. From the cultures that were positive for the presence of this gene, these samples were best characterized by PCR for the presence of other genes cited. The results demonstrated that 5.6% of human isolates were positive for espB EDL933, nleA and espD EDL933 genes; 27.8% were positive for bfp gene and 33% for the gene nleE. As for isolates animals 38.1% were positive for espB EDL933 and nleA genes; 59.5% were positive for nleE gene, and 11.9% were positive for espD EDL933 gene. The results showed that the animals analyzed can be important reservoirs of EPEC, which present virulence genes of the region LEE and non LEE of EPEC. Furthermore, the detection of genes statistically associated with diarrhea in human EPEC, as nleE and espB EDL933, shows that this animals have zoonotic potential.

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P25-11
Why do some proteins evolve slowly?

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Protein evolutionary rates – i.e., the rate by which protein sequences change over evolutionary time, differ by factors of 100–1000, even within the same organism. However, although many plausible functional and biophysical constraints have been examined in relation to evolutionary rates (such as essentiality, dispensability, length, secondary structure, expression level, interacting surface etc.), only weak correlations were found.

We compared not only rates per protein, but also rates per position within individual proteins. For ~90% of proteins, the distribution of positional rates exhibits three peaks: a peak of slow evolving residues, with average log2[normalized rate], log2μ, of ca. -2, corresponding primarily to core residues; a peak of fast

evolving residues ($\log2\mu \sim 0.5$) largely corresponding to surface residues; and a very fast peak ($\log2\mu \sim 2$) associated with disordered segments. However, a unique fraction of proteins that evolve very slowly exhibit not only a negligible fast peak, but also a peak with a $\log2\mu \sim -4$, rather than the standard core peak of -2. Thus, a 'freeze' of a protein's surface seems to stop core evolution as well.

We also observed a much higher fraction of substitutions in potentially interacting residues than expected by chance, including substitutions in pairs of contacting surface-core residues. Overall, the data suggest that accumulation of surface substitutions enables the acceptance of substitutions in core positions. The underlying reason for slow evolution might therefore be a highly constrained surface caused by protein–protein interactions or the need to prevent misfolding or aggregation. If the surface is inaccessible to substitutions, so becomes the core, thus resulting in very slow overall rates.

P25r-12
Atomistic insights on protein-urea structural organization from MD simulations of a chemically denatured protein ensemble determined by NMR

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Urea is a widely used chemical denaturant in protein unfolding studies. However the molecular mechanisms by which it destabilizes proteins remains unclear. MD simulations are ideally suited to provide an atomistic view of protein unfolding induced by urea. Nevertheless, such studies are limited by the accessible time and length scales i.e. urea-unfolding MD simulations are restricted to the observation of partial unfolding events, as well as by the quality of the FFs used. An additional complication is the non equilibrium nature of these simulations, which difficult the validation of the observed mechanism of urea-unfolding with experimental techniques. Here, we overcome some of these limitations by performing explicit solvent full atom MD simulations of a chemically denatured ubiquitin ensemble determined by NMR. Such a strategy allowed us (i) to obtain a reasonable (local) sampling for the unfolded state of ubiquitin in urea (ii) to quantitatively evaluate the quality of the MD simulation against NMR and small angle X-ray scattering data and (iii) to investigate protein-urea structural organization at the end point of the unfolding process. We observed a large enrichment of urea in the first solvation shell of the protein, where urea accumulates preferentially in the vicinity of apolar residues, and forms an increased number of contacts and hydrogen bonds with the protein backbone. However, dispersion interactions arise as the main driving force of urea accumulation around protein and stabilize the unfolded state.

P25-13
Serogroups and virulence genes of pathogenic *Escherichia coli* isolated from commercial one week old layer chicks

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Ninety strains of *E. coli* were isolated from liver and intestine of seven days old commercial layers presenting clinical signs. With the objective of characterizing the isolated strains, the pathogenicity of them was determined by *in vivo* inoculation. The test revealed 44 strains with high and intermediate pathogenicity level, whose were analyzed by PCR for the presence of eight virulence genes (*iss, ast*A, *iuc*D, *irp2, cvi*/*cva, vat, tsh* and*pap*C) and their serogroups were indentified using a set of anti-O antisera. Results demonstrated that these isolated strains contained at least one of the eight genes assessed and the majority of them (93.20%) possessed gene *iss*. Seventeen different genetic patterns have been detected whose 15 had combinations of two or more genes representing 70.45% of all analyzed strains. Eleven different serogroups were identified: O8, O9, O15, O23, O64, O75, O83, O112, O133, O140 and O142. The serogroup most frequent was O8 (15.89%). Eighteen strains did not have their serogroups identified. Results revealed that strains that harbor genes *iss* or *astA* (alone, associated to other genes and associated to themselves) and some that belonged to serogroups O133 and O142 (that have not been reported for APEC group), may have been crucial for the pathogenesis in the studied chicks, once several of these strains had high and intermediate pathogenicity. Obtained results demonstrated the importance of studies in *E. coli* of avian origin in regions engaged in intensive poultry industry, aiming the evaluation of predominant strains and also the acquiring of preventive measures to minimize losses due to colibacillosis.

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P25-14
Towards polarizable molecular dynamics simulations on biologically meaningful timescales: matching additive and polarizable force fields for multiscale simulation

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Molecular dynamics (MD) simulation has increased our understanding of many biochemical processes, but the accuracy of the simulation results is limited by approximations included in the energy functions. Electrostatic interactions, for example, are represented using fixed point charges, omitting the atomic polarizability. The limitations of this approximation are well known, and force fields including explicit representations of polarizability are being developed. Preliminary results are good, indicating that including polarizability yields improved results. But simulations with polarizable force fields are slow, taking 3–10 times as long as those with non-polarizable force fields. With many biochemical processes occurring on timescales at (or beyond) the limit of accessibility with current MD simulations, this slowness limits the applicability of polarizable force fields.

We propose to combine the advantages of polarizable and additive models by using a multiscale approach in which both force fields are employed in a replica exchange scheme, allowing for enhanced sampling with the additive force field. To maximize the overlap between the configurational distributions obtained with the additive and polarizable models, we have used a coarse-graining approach based on the 'relative entropy' to optimize charge parameters for a new additive force field so that it reproduces the configurational distributions of a reference polarizable model. For several small molecules, including water and analogues of biologically important functional groups, the resulting additive force field gives results of similar quality to those obtained with an existing additive force field, while achieving improved overlap with the configurational distributions from the reference polarizable force field. This suggests that as well as facilitating multiscale simulations, coarse-graining based on relative entropy could also provide a general approach for determining charge parameters for additive force fields.

P25-15
Integration of protein-protein docking tools for multi-scale approach to complex structural prediction

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Protein-protein interactions mediate most cellular functions, and the detailed description of their association mechanism is needed to comprehend the fundamental processes that sustain life. Protein-protein docking tools aim to identify the native binding mode between two proteins. Such predictions are required to complement experimental techniques which cannot provide structural information at a proteomics scale given their current technical limitations.

Here we present the integration of a complete set of protein-protein docking tools, developed around the pyDock rigid-body docking framework. Within these tools, we can find: pyDockRST module, which uses the percentage of satisfied distance restraints, together with the electrostatics and desolvation binding energy, to identify correct docking orientations; pyDockPatch, a tool to analyze protein-protein interaction sites and potential hot-spot residues using surface patches; pyDockOPRA, a propensity-based method to identify RNA-binding sites on proteins; pyDockSIPPER, a protein-protein docking scoring based on statistical potentials and precomputed desolvation. The tools will be optimally integrated for the multi-scale application to the structural prediction of protein interactions, and will be available through pyDockWEB, a web server that provides easy access to state-of-the-art pyDock framework for non-expert users.

P25-16
Protein domains as drug targets

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In recent years drug discovery has been driven by the quest for 'magic bullets', drugs that acts selectively on a single target. This assumption has been concurrent with a decrease in the translation of drug candidates into effective therapies (1). The situation, illustrated by the concept of polypharmacology (2), is that there are many drugs for each target and a single drug can affect multiple targets. Network pharmacology is shifting the specific targeted drugs paradigm, in which the dysregulated signalling networks themselves emerge as a new class of targetable entities (3). Most drug targets are proteins that are comprised of domains, their structural and functional building blocks. The interactions between drugs and protein domains can explain the low drug protein specificity.

In this communication we will show our advances in unravelling the interactions between drugs and protein domains, to explore the role of protein domains as drug targets, that can explain drug polypharmacology and can lead to new structure-based target identification and drug discovery strategies.

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P25-17
Transient 3D structure in IDPs ... so what?

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Intrinsically disordered proteins (IDPs) play fundamental roles in biology and are commonly associated with human diseases. Under native conditions, IDPs contain long unstructured regions in their sequence, which can account for the full protein. However, IDPs are not random-coil polymers, as they contain transiently formed secondary and tertiary structural patterns. Are these low populated structural residual patterns functionally relevant? Here by examining the structural and evolutionary features of IDPs we will provide evidence on the functional relevance of transiently formed structural patterns.

P25-18
Flexible protein-protein docking with discrete molecular dynamics

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Protein-protein interactions are responsible for the transfer of information inside the cell and currently represent one of the most interesting research fields in structural biology. However, experimental approaches have difficulties in providing 3D structures for the specific interactions formed between the different proteins in a living organism. The use of theoretical approaches like docking aims to complement experimental efforts, but there are cases especially difficult for prediction, those in which complex formation implies a non-negligible change in the conformation of the interacting proteins, i.e. those cases where protein flexibility plays a key role in protein-protein docking. In this work we present a new approach to introduce flexibility in docking by global structural relaxation based on ultra-fast discrete molecular dynamics. On a standard benchmark of protein complexes, the method provides a general improvement over the results obtained by rigid docking.

P25r-19
Exploring conformational selection mechanism in protein-protein association by docking

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Protein-protein interactions are essential for the majority of protein functions. Thus, to understand life processes at molecular level we need a deep structural knowledge of the hundreds of thousands of protein-protein complexes that are formed in the cell. However, experimental determination of 3D complex structures by atomic resolution techniques like NMR and X-ray crystallography is not keeping pace with the genomics information generated by sequencing projects. To complement experimental efforts, a variety of computational tools have been reported to predict a complex structure by docking. The CAPRI experiment

has shown the success of different docking approaches (1). Among them, our method pyDock (2) showed that correct predictions are often possible based on exhaustive rigid-body search and effective energy-based scoring function. However, the performance drops dramatically for cases with large conformational changes upon binding (3). Thus, the major challenge in protein-protein docking remains the appropriate treatment of molecular flexibility and conformational changes. From the conformational selection hypothesis, we should expect that precomputed unbound ensembles could include bound conformers that could be successfully used in rigid-body docking. This is a potentially useful strategy to include flexibility in docking that has been rather unexplored so far (4). Here we will present a systematically study of docking with conformational ensembles generated from the unbound proteins by using three different computational approaches: modelling minimization tools (MM), Molecular Dynamics simulations (MD) and Normal Mode Analysis (NMA). In order to evaluate the suitability for docking of each conformational sampling method, we selected the best possible conformers from each ensemble, i.e. those with the lowest values of bound-unbound Ca-RMSD, interface RMSD and optimal docking binding energy, respectively. Finally we used them to perform pyDock rigid-body docking simulations for each complex. The use of MM and MD ensembles improves the general predictive results on a standard benchmark of 124 protein-protein complexes (5), especially in those cases with intermediate conformational changes. We also show that conformers automatically generated through a combination of normal modes with the MRC server (6) (http://abagyan.ucsd.edu/MRC/) can significantly improve the rigid-body results in highly difficult cases with large conformational flexibility upon binding. These results bring interesting discussion ideas on the protein-protein association mechanism (conformational sampling versus induced fit). The methodological challenge is now how to use the capabilities of unbound conformational ensembles in an efficient docking algorithm.

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P25r-20
Unraveling the human interactome: construction and analysis of protein-protein networks at different confidence levels and with different types of information

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Decades of protein biochemistry studies have produced a remarkable compendium of knowledge about the functional properties of many individual human proteins. Nowadays large-scale proteomic techniques are providing a novel insight into the complex atlas of proteins that work in different human cell types in different biological states. Proteins do not act alone, but rather team up into molecular machines and macromolecular structures enclosing intricate interaction networks to undertake biological functions. A key step towards unraveling the complex molecular relationships in a living system is the mapping of protein-to-protein physical 'interactions' (De Las Rivas and Fontanillo, 2010). Such map has been called the 'interactome'. To achieve a complete atlas of the protein interactions within an organism will allow to build its interaction network and to identity the critical nodes that are essential for the functional survival of such organism. Focusing on human, the interactome can be most relevant for current biomedical research, because the location of a given protein in the interaction network will allow to evaluate its centrality and its contribution to the performance and regulation of specific biomolecular pathways. In fact, many diseases can be defined as an altered state in the communication pattern of the proteins acting in the cells or tissues that suffer such pathological state (Barabasi et al., 2011). We present in this report our most recent studies to characterize a comprehensive binary human interactome, to identify protein interaction subsets associated to specific biological pathways and to correlate human interactome topology with defined biological functions (Souiai et al., 2011).

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P25-21
Exploiting protein flexibility to predict allosteric sites

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Protein allosteric sites are increasingly attracting the interest of medicinal chemists in the search for new types of targets and strategies to drug development. Given that allostery represents one of the most common and powerful means to regulate protein function, the traditional drug discovery approach of targeting active sites can be extended by targeting allosteric or regulatory protein pockets that may allow the discovery of not only novel drug-like inhibitors, but activators as well (1).

Moreover, allosteric sites present additional characteristics, such as modulable activity and less evolutionary pressure, which may facilitate the development of highly-specific allosteric drugs that can prevent side effects and readily complement traditional therapeutics.

Continuing with our previous work on allosteric sites from a structural and evolutionary perspective (2), we have now developed a method to predict the location of allosteric sites on protein structures.

The methodology, which is a coarse-grained approach based on protein flexibility, achieves up to 65% accuracy on a set of 58 different protein families. To our knowledge, this work represents the first attempt to predict allosteric sites on multiple protein families.

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Transgenesis in Mammals

P26-1
Conditional deletion of insulin-like growth factor 1 receptor in the lung epithelium of mutant mice

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Insulin-like growth factors 1 (IGF1) and IGF2 can influence growth, differentiation, and survival of cells expressing their cognate type 1 receptor (IGF1R). IGF genes have been involved in both, normal lung development and lung repair after injury. They are also implicated in relevant respiratory diseases such as respiratory distress syndrome, idiopathic pulmonary fibrosis and lung cancer. IGF1R- and IGF1-deficient mice die at birth because their lungs are disproportionally hypoplastic and collapsed, with alterations in alveolar epithelium maturation and vasculogenesis. Furthermore, reduction in IGF1R signaling confers resistance to lung airway damage and protects against pulmonary fibrosis and oncogenesis. Despite its relevance, *in vivo* cell autonomous IGF1R function in the epithelial compartment of the lung is poorly known. To better understand IGF1R signaling in lung airway and alveolar type 2 epithelial cells, cell types that express the highest levels of IGF1R in the lung, we generated two conditional (Cre/loxP) lung epithelium-specific IGF1R knockout mouse models. By using a transgenic line that targets Cre recombinase to the airway Clara cells (*CCSP-Cre*) in *Igf1r floxed* mice with low efficiency, conditionally mutant animals developed to adulthood and their lungs revealed no abnormalities in size, morphology, histology or ventilatory function. An *Nkx2.1-Cre* transgenic line, that drives Cre to the airway and alveolar type 2 epithelial cells with high efficiency, it is currently in use to get an alternative way of conditional deletion of IGF1R in these cell types. Preliminary results on lung epithelium proliferation, epithelium repair after naphthalene injury or *in vitro* epithelial stem cell differentiation analyses in both mutant models will be shown and discussed.

P26-2
Pleiotropic effects of rosuvastatin on inflammation, oxidative stress, insulin resistance and target organ damage in CRP transgenic spontaneously hypertensive rats

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The JUPITER trial suggested beneficial effects of rosuvastatin on cardiovascular disease risk in individuals with higher levels of C-reactive protein (CRP). Recently, we derived 'humanized' spontaneously hypertensive rats (SHR-CRP) in which transgenic expression of human CRP induces inflammation, oxidative stress, several features of metabolic syndrome and target organ damage. In the current study, we tested the effects of rosuvastatin (10 mg/kg) in SHR-CRP males versus untreated controls. All rats were fed a high sucrose diet. In SHR-CRP transgenic rats, treatment with rosuvastatin for 10 weeks, compared to untreated transgenic controls, was associated with significantly reduced endogenous CRP but not transgenic CRP and with amelioration of inflammation and oxidative stress. In addition, rosuvastatin treatment was associated with significantly reduced epididymal and perirenal fat deposits and decreased hepatic triglyceride levels, reduced plasma triglycerides and insulin, and higher sensitivity of adipose tissue to insulin action. Furthermore, statin treated rats showed significantly reduced microalbuminuria suggesting amelioration of renal function. These findings provide evidence for important antiinflammatory, antioxidative, hypolipidemic and antidiabetic effects of rosuvastatin. Analyses of gene expression profiles revealed molecular mechanisms underlying anti-inflammatory effects of rosuvastatin.

P26-3
Inherited variation in mtDNA in SHR-mt^{F344} versus SHR conplastic strains is associated with reduced OXPHOS enzyme levels, insulin resistance, and left ventricular hypertrophy

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Common inbred strains of the laboratory rat can be divided into four major mitochondrial DNA (mtDNA) haplotype groups represented by the SHR, BN, LEW and F344 strains. In the current study, we investigated the metabolic and hemodynamic effects of the SHR versus F344 mitochondrial genomes by comparing the SHR versus SHR-mt^{F344} conplastic strains that are genetically identical except for their mitochondrial genomes. Altogether 12 amino acid substitutions in protein coding genes and seven single nucleotide polymorphisms in tRNA genes were detected in F344 mtDNA when compared to SHR mtDNA. Analysis of oxidative phosphorylation system (OXPHOS) in heart left ventricles (LV), muscle and liver revealed reduced activity and content of several respiratory chain complexes in SHR-mt^{F344} conplastic rats when compared to the SHR strain. Lower function of OXPHOS in LV of conplastic rats was associated with significantly increased LV mass (0.224 ± 0.014 versus 0.159 ± 0.005 g/100 g BW, $p = 0.0006$) which was independent on blood pressure. In addition, conplastic rats exhibited reduced sensitivity of skeletal muscles to insulin action and impaired glucose tolerance. These results provide evidence that inherited alterations in mitochondrial genome, in the absence of variation in the nuclear genome and other confounding factors, predispose to insulin resistance and left LV hypertrophy.

P26-4
Hormone-sensitive lipase deficiency in mice alters sterols and enzymes involved in cholesterol synthesis in the testis

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Dyslipidemia, obesity and metabolic syndrome have been related with infertility. There is a tight relationship between fertility and changes in cholesterol metabolism during spermatogenesis. Cholesterol and other sterols are necessary to spermatogenesis and steroidogenesis, and they are essential for the germinal cells development. Hormone sensitive lipase (HSL) is an intracellular neutral lipase that catalyzes the hydrolysis of diacylglycerides, cholesterol and retinoid esters, it is reasonable to think that cholesterol released by the action of HSL is required for spermatogenesis. The role of HSL in testis was revealed by the phenotype of HSL-knockout (KO) mice. HSL KO male mice are sterile.

Objetive: The aim of this study was to investigate the role of lipids in fertility by studying the effect of the lack of HSL in lipid and sterol composition, and mRNA expression of the enzymes involved in sterols metabolism in mice testis, in order to get an insight on the mechanisms mediating the alterations in spermatogenesis and ultimately infertility in these animals.

Methods: We have measured the sterol composition by GC/MS; mRNA expression by real time RT-PCR; and cellular distribution of neutral lipids by Oil Red and free cholesterol by Filipin staining of histological slices of WT and HSL-KO mice testis.

Results: In the HSL-KO testis compared with WT; we have found a significative increased in plasma cholesterol and neutral lipids contents into Leydig cells, without changes in the triglycerides and testosterone plasma concentrations. In parallel we show an increase of cholesterol, 4α-metilcolesta-8(9)-en-3β-ol, lathosterol and desmosterol and alteration in the enzymes HMGR, SC4MOL, DHCR24, SREBF2, 14α-demethylase, and Δ8,7-isomerase in HSL-KO mice testis.

Conclusions: The lack of HSL cause alterations in lipid metabolism, accumulation of cholesterol, changes in the sterol composition and the enzymes involved in cholesterol synthesis. These changes could contribute or to be responsible of the infertility in HS-KO mice.

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P26-5
Dlk2 overexpressing mice show premature aging and loss of adipose tissue in the adulthood

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DLK2 is a transmembrane glycoprotein closely related to DLK1. Both proteins possess six epidermal growth factor-like (EGF-like) motifs in their extracellular domain that interact with the NOTCH1 receptor and are able to inhibit its activation and signaling, thus modulating cell fate differentiation. Adipogenesis is a differentiation process in which both DLK1 and DLK2 are involved. In fact, *Dlk1* KO mice show growth retardation and an increase in the number and the size of adipocytes in the adulthood. To investigate the role of *Dlk2 in vivo*, here we describe the generation and initial characterization of a mouse model ubiquitously expressing *Dlk2*. The animals, named Dlk2TG, expressed high levels of *Dlk2* mRNA in all tissues analyzed. Dlk2TG mice showed a clear impairment for normal reproduction and a shorter lifespan (55 weeks as compared to 120 weeks of their WT counterparts). Around 65% of *Dlk2TG* animals died of heart failure, showing cardiac hypertrophy, abundant pericardic infiltrations, clotting lesions and edema in the myocardium. During the first weeks of life, the WT and *Dlk2TG* mice were phenotypically very similar. However, as Dlk2TG mice grew older they developed what could be considered as a premature aging process, with a pronounced kyphosis of the spine, associated to gradual loss of fat deposits in different locations of the body. Although the size, weight and body mass index of *Dlk2TG* animals was not significantly different from WT animals, *Dlk2* transgenic mice showed smaller abdominal adipose pads and less accumulation of fat around the kidneys, aortic and mesenteric locations. Our results indicate that *Dlk2TG* animals suffer of a premature aging process, accompanied by an impaired development of adipose tissue in the adulthood.

P26-6
EMMA: The European Mouse Mutant Archive

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The European Mouse Mutant Archive (EMMA) is a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mouse mutant strains essential for basic biomedical research. The EMMA network is a partnership of 14 laboratories and other institutions throughout Europe. CNB-CSIC in Madrid hosts the Spanish EMMA node.

EMMA's primary objective is to establish and manage a unified repository for maintaining biomedically relevant mouse mutants and making them available to the scientific community. Therefore, EMMA archives mutant strains and distributes them to requesting researchers. At present, EMMA holds over 3000 mouse strains, corresponding to transgenic mice, different type of mutants, gene-traps, knock-ins, knock-outs and also including some targeted alleles from Deltagen, Lexicon and EUCOMM projects. EMMA's technology development programme is focusing on improving sperm cryopreservation methods, the implementation of laser-assisted IVF and ICSI protocols, and has explored different techniques to allow the shipment of unfrozen mouse embryos. Drivers of a continued growth of the EMMA

archive are large scale programs such as IKMC, the Sanger Mouse Genetics Project and EUCOMMtools. These developments necessitate continued efforts in technology development, capacity building and securing sustainable funding.

EMMA also hosts cryopreservation courses, to promote the use and dissemination of frozen embryos and sperm. All EMMA procedures and all required information to deposit or request mouse lines from EMMA are easily available through the EMMA web site at: www.emmanet.org.

EMMA is supported by the partner institutions, national research programmes and by the EC's FP7 Capacities Specific Programme.

P26-7
Transcriptome resources for the complexity of developing mouse brain: from Eurexpress to Eucommtools through Allen Brain Atlas

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When and where genes are expressed in brain is not only crucial to understanding and/or predicting the physiological role of genes and proteins but also to get insight into how they interact to form the complex networks that underlie brain development and function.

Several transcriptome-wide acquisitions of gene expression patterns propose high-resolution scale (cytoarchitecture) by means of in situ hybridization (ISH) with non-radioactive probes to establish a web-linked, interactive digital transcriptome atlas of embryonic mouse brain. These 'transcriptome atlases' are generated using an automated RNA in situ hybridization system. Automated scanning microscopes are then collected the images data, which will be electronically sent or linked in a digital format for anatomical annotation. The latter will be performed using a web-based 'virtual' microscope and be entered in a hierarchical database specifically designed to hold large amounts of image data and display them in a user-friendly format. The final goal of these projects is to create an open website gene expression database resources of thousands genes available for the scientific community.

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P27 – Others

P27-1
MMP-3 and E-Cadherin polymorphisms in breast cancer

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Breast cancer and other malignities are caused by genetic alterations affecting cellular pathways participating in cell growth and development. Cadherins are connected each other by homophilic binding and they play important roles in cell adhesion, ensuring that cells within tissues are bound together. Because of the irregular behavior of tumor cells to cell-cell relationship is corrupted. The main characteristic of invasive carcinoma cells are they are poorly differentiated and their activity is increased. E-cadherin leads to the destruction of property of the cell mobility. In breast cancer issues, due to E-cadherin expression or disorder in phosphorylation of cytoplasmic part of epithelial cells, they gains mobility. The lack of cadherin is responsible for emergence of invasive phenotype and ductal carcinomas, leading to a poor prognosis. MMP gene family, codes 9 or more endopeptitase against extracellular matrix macromolecules. It has been reported that increased MMP-3 expression in breast cancer is encouraging tumor prognosis. In this study, we aimed to investigate the effect of the E-Cadherin -160 C/A and MMP-3 5A/6A gene polymorphisms in breast cancer. Our study included 65 breast cancer patients and 54 age-matched controls. Clinical parameters for both patients and controls were collected from the hospital records. To determine relevant gene polimorphisms we used PCR-RFLP method. As a result of statistical consideration, we find out that carrying 6A allel of MMP-3 gene and C allel of E-Cadherin can play a role in disease progression and metastasis.

P27-2
Effect of anoxia on protein synthesis process in the isolated pig heart

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An insufficient supply of oxygen to tissues influences protein synthesis in different levels of this process. The aim of this study is to investigate what components of the cell-free protein synthesis system are influenced by anoxia. The effect of anoxia was evaluated after 20 and 90 min of anoxic perfusion. For the determination of the intensity of protein synthesis, the incorporation of [^{14}C]-leucine into translational products in a cell-free system was measured.

The protein synthesis level decreased by 19% in the cell-free system containing cytosol and ribosomal fraction from the anoxic heart as compared with the control heart. The analogous results were observed when the cell-free system contained cytosol from the anoxic heart and ribosomal fraction from the control heart. When translation system containing cytosol from the control heart and ribosomal fraction from the anoxic heart was used, the results were the same as in the control heart. In the case of the 90 min anoxia the protein synthesis level diminished by 45% when cytosol from the anoxic heart was used and only by 16% when ribosomal fraction from the anoxic heart was used.

The protein synthesis level in the cell-free translation system under oxygen deprivation is related to changes in cytosol and ribosomal fraction. The decrease of the protein synthesis level in a cell-free translation system under anoxia correlates with the decrease of the tRNA and aminoacyl-tRNA synthetases activities under the same conditions. Consequently, we can conclude that one of the causes influencing protein synthesis process in a cell-free translation system under anoxia may be the changes in the tRNA and aminoacyl-tRNA synthetases activity under anoxia.

P27-3
Hepatitis A virus contamination in the river and soil of Kitakyushu area in Japan

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Hepatitis A virus (HAV) is classified as a picornavirus and one of the major causes of viral hepatitis. The annual incidence of hepatitis A is 1.5 million cases of clinical disease, and the true incidence, including subclinical disease, may be much higher. After ingestion uptake in the gastrointestinal tract, and subsequent replication in the liver, HAV is excreted in bile, and high concentrations are found in stool specimens. Transmission occurs by the fecal-oral route, either by direct contact with an HAV-infected person or by ingestion of HAV-contaminated food or water. For most persons, HAV lasts for several weeks. Relapsing symptoms, accompanied by renewed elevation of serum aminotransferase levels, occur in 10% of cases, and relapses might continue for as long as 6 months. The overall case-fatality rate is 0.3%, but it is 1.8% among persons age >50 years. Persons with underlying chronic liver disease have an increased risk of death. The incidence of HAV in Japan has markedly decreased recently. National surveillance of HAV in Japan has shown that more than 90% of people over 65 but fewer than 10% of people under 34 are positive for anti-HAV. Good sanitation and a sterilized water supply are essential for the prevention of HAV. In developing countries with a high incidence of HAV, the main transmission route of HAV is the fecal-oral route caused by poor sanitation. In this report, we tried to investigate the HAV contamination of the river, sea water and soil in suburbs of kitakyushu area, Japan.

P27-4
Identification of novel molecular determinants of tissue mineralization in fish

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Understanding the biology of tissue calcification to prevent health problems such as osteoporosis is a major challenge for the next decades. In this context, various cell systems capable of *in vitro* mineralization have been developed to identify novel genes involved in this process. Three genes differentially expressed in a fish (gilthead seabream) pre-osteoblast cell line undergoing ECM mineralization, have been further evaluated: SDR coding for a short-chain dehydrogenase reductase, S100 coding for a calcium-binding protein and BHMT3 coding for a betaine homocysteine methyl transferase. Analysis of the spatial-temporal expression of these three genes by qPCR and *in situ* hybridization revealed: (i)

the up-regulation of SDR transcript during *in vitro* mineralization and its specificity for calcified tissues, in particular the bone opercula and osteoblast therein. Expression observed in the stomach indicated that SDR is probably involved in other physiological processes; (ii) the up- and down-regulation of S100 and BHMT3 genes, respectively, during *in vitro* mineralization of seabream cell lines and during seabream development, from larvae throughout adulthood. Expression of both genes was restricted to chondrocytes of cartilaginous tissues undergoing endo/perichondral mineralization in juvenile fish. Expression data collected *in vitro* and *in vivo* for SDR, S100 and BHMT3 suggest a role for these three proteins in mechanisms of mineralization in fish – roles that remain to be determined – and emphasized their potential as markers of mineralizing cartilage in developing fish. The transcriptional regulation of the three genes is currently investigated through the functional analysis of gene promoters *in vitro* (using luciferase reporter constructs) and *in vivo* (using β-galactosidase reporter constructs).

P27-5
The expression and activity levels of RhoC, ROCK-I, ROCK-II and MMP-2 in colorectal cancer

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Most of deaths derived from colorectal cancer (CRC) are due to metastasis rather than primary tumor; therefore CRC is the fourth leading cause of cancer death after lung, prostate and breast cancers. Matrix metalloproteinases (MMPs), which are related to cancer progression, contribute to metastasis formation by removing extracellular barriers. In addition, RhoC and ROCK proteins involved in cell migration are also thought to be associated with CRC progression and metastasis. We aimed to investigate MMP-2, RhoC, ROCK-I and ROCK-II expression and activity levels in tumor and paired normal tissue samples taken from CRC patients and compare the results with clinicopathologic variables. For this purpose in colorectal tumor and paired normal tissue samples (n = 23), protein levels of MMP-2, RhoC, ROCK-I and ROCK-II were detected with Western Blot and MMP-2 and ROCK-II activity levels were determined with Gelatin Zymography and ROCK-II Activity Assay, respectively. There were no statistically significance in MMP-2, RhoC, ROCK-I and ROCK-II protein expression levels and ROCK-II activity between tumor and paired normal tissues. However, activeMMP-2 levels ($p = 0.002$) and activeMMP-2/proMMP-2 ratio ($p = 0.015$) were found significantly elevated in tumor tissues compared to paired normal tissues. We also found significant correlations between ROCK-II activity and tumor staging ($p = 0.006$) and activeMMP-2/proMMP-2 ratio and pathological staging ($p = 0.007$). We predict that elevated levels of active MMP-2 and ROCK-II increases the ability of CRC invasion and promotes metastasis through activation of proteolysis-dependent mechanism. Therefore not only MMP inhibitors but also ROCK inhibitors may be selected as target molecules in the use of new chemopreventive therapy for individual CRC patient.

P27-6
Identification and characterization of four novel p60 katanin-like proteins (Katnal2) in mouse

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Katanin is a microtubule-severing AAA protein. Several organisms encode a number of Katanin-like proteins that are similar to but different from canonical Katanin and whose cellular functions are largely unexplored. We have identified and cloned four novel Katanin-like 2 proteins (Katnal2) from mouse NSC34 neuroblastoma cells (unpublished). Even though these proteins are closely related to Katanin, they remain uncharacterized. The mRNAs of these isoforms have different sizes (1617, 1611, 1215 and 1116 bp) and are products of differential mRNA splicing. They encode four distinct but highly similar isoforms (Katnal2 L1, L2, S1 and S2) that exhibit an ATP/GTP binding motif A (P-loop). A LiSH domain is present in the two larger isoforms (L1, L2) but absent in the other two isoforms (S1, S2).

Differential expression studies by RT-PCR revealed that the three larger isoforms are widely expressed across many of the mouse tissues examined; however their pattern of expression is distinctly different. The expression of the smallest isoform is more restricted with notable enrichment in the hippocampus. The expression of the larger isoforms appears to be developmentally regulated in the mouse embryo.

Using an anti-peptide antibody designed to recognize all four isoforms, we showed in NIH3T3 mouse cells that at interphase these isoforms are expressed throughout the cell in both nucleus and the cytoplasm, with more intense staining in the nuclear envelope region. In mitotic cells immunoreactivity localizes at the centre of the two microtubule asters at the beginning of mitosis (up to prometaphase), and extends to spindle microtubules in later mitotic stages.

Silencing experiments with the use of isoform-specific siRNAs were performed, in order to assess mitotic and interphase phenotypes associated with the lack of expression for each of the Katanin-like isoforms, without success. That led to the use of shRNA for stable silencing experiments, which are currently in progress.

Immunoprecipitation experiments are currently being pursued to reveal possible interacting partners, while microtubule severing assays are also carried out towards the functional characterization of the Katanin-like 2 isoforms.

P27r-7
A SUMO-dependent step during establishment of sister chromatid cohesion

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Cohesin is a protein complex that ties sister DNA molecules from the time of DNA replication until the metaphase to anaphase transition. This molecular connector is essential for correct chromosome segregation and to maintain genome integrity. Its activity is therefore controlled by several post-translational modifications, including acetylation, phosphorylation and site-specific proteolysis. Here we show that cohesin is also regulated by the small ubiquitin-like modifier (SUMO). Our molecular dissection indicates that cohesin sumoylation occurs at the time of cohesion establishment, after cohesin loading and ATP binding, and independently from Eco1/Ctf7-mediated cohesin acetylation. In order

to test the functional relevance of cohesin sumoylation, we have developed a novel approach to deplete SUMO from all subunits in the cohesin complex, based on fusion of the Scc1/Mcd1/Rad21 subunit to a Ulp SUMO-peptidase domain (UD). Down-regulation of cohesin sumoylation is lethal, and the Scc1-UD chimeras have a failure in sister chromatid cohesion. Strikingly, the unsumoylated cohesin rings are acetylated. Our findings indicate that SUMO is a novel molecular determinant for the establishment of sister chromatid cohesion, and we propose that SUMO is required for the entrapment of sister chromatids during the acetylation-mediated closure of the cohesin ring.

P27-8
Synergistic action of antibiotics and bacteriophages on bacteria

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Antibiotic resistance is a major public health concern, as well as in agriculture and food safety. The growth of pathogen microbes in particular the drug-resistant forms renders more difficult to fight against infectious diseases. The half-life of antibiotics is becoming so short that pharmaceutical majors hesitate to develop new bactericidal molecules that cost years of development. An effective alternative could be the use of cocktails of sub lethal doses of these molecules with alternative therapy such as lytic phages.

Our results show that several combinations of phages with various classes of antibiotics used at sublethal dose can inhibit bacterial growth whereas they do not if used independently. Experiments conducted with temperate phages such as HK620 have shown a synergistic action of these phages subinhibitory concentrations with small amounts of antibiotics. This effect was observed with several phage/antibiotic combinations, but not all tested.

Our results suggest that temperate phages as well as lytic could be effective elicitators for infectious disease treatment when combined with antibiotics at low concentrations.

P27-9
Isolation, characterization and functional analysis of a new nitric oxide synthase (NOS) gene in the lobster *Panulirus argus*

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Nitric oxide (NO) is a free radical gas involved in a variety of physiological processes in invertebrates. Surprisingly, little is known about the involvement of NO synthase (NOS) in the immune system of crustaceans. This work is focused on the study of the NOS gene of the spiny lobster *Panulirus argus*, and its relationship with the immune response. A NOS full-length DNA was isolated from hemocytes by reverse transcription-polymerase chain reaction (RT-PCR). The open reading frame (ORF) encodes a protein of 1200 aa, with an estimated molecular mass of 135.9 kDa. NOS gene expression in lobster tissues was studied by Real Time qPCR and was higher in hemocytes, heart and gills. In addition, when lobster hemocytes and gills were exposed to *Escherichia coli* O55:B5 lipopolysaccharide (LPS), an increase in the NOS activity and also in the NOS gene expression was observed. The 3D NOS structure was predicted by comparative modeling, allowing the selection of a fragment of 666 bp that was cloned and subsequently expressed in *E. coli* BL21, in which a recombinant product of 31 kDa was obtained. Hyperimmune serum obtained from immunized rabbits was tested and employed to specifically detect the endogenous NOS from lobster hemocytes by western blot and immunofluorescence. Additionally, the antibacterial activity of the hemolymph of the shrimp *Litopenaeus vannamei* decreased after the immunoneutralization of NOS. These results demonstrate the presence of an inducible crustacean NOS, that will be useful for evaluating the immunological response to bacterial infections in these organisms.

P27-10
Catalytic versatility of DNA cytosine-5 methyltransferases: reactions involving non-cofactor-like substrates

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DNA cytosine-5 methyltransferases catalyze site-specific transfers of a methyl group from the cofactor S-adenosyl-L-methionine (SAM) onto the 5-position of their target cytosine residues in DNA. Recently we have shown that, in the absence of SAM, methyltransferases are able to add formaldehyde to their target cytosines yielding 5-hydroxymethylcytosine (hmC) (1). This reaction can be reversed yielding unmodified cytosine, or can be further extended by condensation with thiols or selenols (2). Lately hmC was discovered in mammals DNA (3) but the biological role of this new base is unclear because further studies are restricted by the lack of efficient analytical techniques for mapping hmC residues in the genome. These atypical reactions of DNA cytosine-5 methyltransferase open new ways for analysis of hmC in genomic DNA and provide inroads into active demethylation of 5-methylcytosine residues in the genome.

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P27m-11
Ligand-detected ^{19}F NMR-based fragment screening for drug discovery against selected macromolecular targets

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In the last decade an alternative approach to molecular target-focused drug discovery based on high throughput cell or biochemical assays, termed fragment-based drug discovery, has gained widespread interest and application. It is based on the idea of building drugs piece by piece, detecting first the weak binding of low molecular weight (typically MW < 150–300 Da) and low complexity compounds (fragments), that could subsequently be chemically developed into more complex molecules or combined or linked with other fragments that bind in an adjacent

site of the macromolecular surface, thus resulting in compounds with lead-like properties. The approach is particularly promising to target protein-protein interactions. In our Unit we have developed tools to conduct NMR-based screening to identify and characterize the binding of small molecules to protein targets, potentially providing hits for drug discovery research and chemical tools for biophysical and functional applications. In particular, we put together a collection of ~370 fluorinated small molecule compounds with good solubility in aqueous buffer and assembled them into mixtures of eight each. Using 1D ^{19}F NMR spectroscopy we were able to monitor the possible binding of the compounds present in each cocktail to different protein targets by monitoring the increase of ^{19}F NMR signal linewidth upon protein addition. Typically 20 μM concentration in each CF_3-containing fragment, or 50 μM for those with CF groups, are sufficient to obtain high quality spectra, and as low as 1:50 of protein equivalents produce a detectable effect on fragment binders, therefore requiring very small amounts of protein. With a sample changer and a dual H-F probe in our 700 MHz instrument, screening of the complete fluorinated fragment library can be performed in < 2 days. Results obtained in the initial screening and the follow up of hits discovered against selected macromolecular targets will be presented in this poster.

P27-12
Safety test of canine adipose-derived stem cells

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Recent scientific achievements in the cell and molecular biology have promoted development of autologous stem cell therapy offering comprehensive possibilities for the treatment of human and animal diseases and dysfunctions. Before the clinical use of stem cells, safety tests must be performed at first.

Adipose tissue from a Beagle dog was used to isolate adipose-derived stem cells (ADSCs) that were cultivated and successfully propagated in a medium supplemented with the autologous serum. After the second passage cells were frozen and then thawed for characterization and future examination. Fraction of obtained ADSCs was cultivated under 5% oxygen tension (ADSCsA) and were characterized in contrast to cells cultured under 21% oxygen (ADSCsB).

Both types of cells were plastic adherent spindle-shaped cells with fibroblastoid morphology. Telomeres of ADSCsA were increased by 12.4% and expression of surface marker genes CD73, CD90, CD105, CD34 and pluripotency gene NANOG was higher than in ADSCsB. Immunomodulatory properties of ADSCs A and B were compared by blast transformation reaction using dog's peripheral blood mononuclear cells. Significant suppression of T lymphocyte proliferation was induced in a dose dependent manner by both types of cells, but ADSCsA showed more pronounced immunosuppressive effect than ADSCsB.

To test the safety of ADSCs cultured under 5% oxygen, the therapeutic dose of ADSCsA (1×10^6 live cells/kg) were injected into the dog's bloodstream followed by the repeated injection of five times higher dose after a month. No significant side effects were observed after both experiments and all the tested physiological, biochemical and blood circulation parameters were within normal limits testifying towards the therapeutic safety of canine ADSCs.

P27-13
Engineering copper hyperaccumulation in plants by expressing a prokaryotic *copC* gene

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Copper is an essential microelement in plants, acts as cofactor in enzymes and is involved in the synthesis of chlorophyll. However, high concentrations of Cu become toxic to the cells, generating free radicals that damage DNA and membranes. Biotechnology offers phytoaccumulation strategies as a potential tool in the bioremediation of copper polluted soils.

In this work, several genetic strategies have been approached for the development of Cu-hyperaccumulation in plants. First, the *copC* gene from *Pseudomonas fluorescens*, encoding a periplasmic Cu-binding protein, has been expressed in *Arabidopsis thaliana* plants under the control of the constitutive CaMV 35S promoter (transgenic lines 35S-copC). 35S-copC lines showed up to 5-fold increase Cu accumulation compared to wild type untransformed plants, both in roots (up to 2000 μg Cu/g) and shoots (up to 400 μg Cu./g), over the limits recently established for Cu-hyperaccumulators. 35S lines showed slightly enhanced Cu sensitivity. Secondly, copC was engineered under the control of the *cab1* (chlorophyll a/b binding protein 1) promoter, in order to drive *copC* expression to shoots (transgenic lines cab1-copC). Cab1-copC lines showed increased Cu translocation factors (twice that of wild type plants). Finally, subcellular targeting of the CopC protein to plant vacuoles was addressed by expressing a modified *copC* gene containing specific vacuole sorting determinants (transgenic lines 35S-copC-V). Transgenic 35S-copC-V lines did not show further increased Cu-accumulation, -neither in roots nor in shoots-, when compared to 35S-copC lines. Conversely, they did display enhanced Cu-hypersensitivity. Our results demonstrate the feasibility of obtaining Cu-hyperaccumulating plants by engineering a prokaryotic Cu-binding protein.

P27-14
Identification of novel chromatin modulators of the DNA damage response

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Cells are constantly under attack by DNA damaging agents, arising from both exogenous and endogenous sources. DNA double-strand breaks (DSBs) are particularly deleterious, as an error in repairing them can lead to cell death or genome instability, the latter of which can directly cause cancer. Cells have therefore developed an elaborate signalling cascade, the DNA damage response (DDR) that, via phosphorylation and recruitment of a plethora of proteins to DNA damage sites, maintains genome stability.

While the influence of chromatin structure on transcription and DNA replication is well established, its importance for the DDR has only recently been realized. To identify novel chromatin factors involved in the DDR, I have recently performed a high-throughput, high-content siRNA screen in mammalian cells. After knockdown of more than 300 chromatin factors, the cells were challenged with DSBs and stained against the early DDR marker gammaH2AX and the later marker 53BP1. Analysis of these DDR fingerprints revealed several candidate genes involved in the regulation of histone three lysine 36 (H3K36) methylation, and the role of these factors in the DDR will be discussed.

P27-15
Comparative kinetic study of lipases from *Burkholderia cepacia, Rhizomucor miehei* and *Candida rugosa* in esterification reactions in organic solvent

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Lipases (glycerol ester hydrolases EC 3.1.1.3) are widespread in nature and have been shown to catalyze hydrolysis/synthesis of wide range of soluble and insoluble carboxylic acid esters and amides. Among the enzymes studied to date, lipases are most attractive due to their numerous biotechnological applications in the preparation of fine chemicals, food and pharmaceutical industry. Catalytic action of lipases is rather complex and the shape and structure of the enzyme around the active site varies significantly from one lipase to another (1,2). In the present work, lipases with different structures were investigated for their catalytic ability to esterify oleic acid with butanol in isooctane. Besides, the kinetic behavior of these enzymes was studied. The lipases used were from *B. cepacia* (BCL) supplied by *Amano*, *C. rugosa* (CRL) by *Sigma* and *R. miehei* (RML) provided by *Novozymes*. Firstly, the effect of enzyme concentration on the esterification rate was studied with optima at 30 mg CRL/ml, 40 ml RML/ml and 30 mg BCL/ml. The lipase from *R. mihei* evidenced the highest activity with acid oleic as substrate. Kinetic studies showed that the reaction follows a Ping-Pong Bi Bi mechanism with inhibition by excess of n-butanol for all the lipases. The values of all apparent kinetic paramaters (V_{max}, K_m acid and K_m alcohol) were also determined.

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P27-16
Effect of external voltage on proteins appearance in *Pseudomonas putida* grown in bio electrochemical cell

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A Bio Electrochemical Cell (BEC) was constructed as a typical two-chamber microbial fuel cell (MFC), except that it was operated under external voltage instead of constant resistances as in a MFC. The anode chamber contained a pure culture of *Pseudomonas putida* F1 grown in a minimal medium containing toluene as the sole carbon and energy source. Operating the BEC under an external voltage of 75, 125, 175, 250 and 500 mV (versus Ag/AgCl) led to increased bacterial cell growth to 0.62–0.75 $OD_{600\ nm}$, respectively, while the control BEC which was not connected to external voltage, reached 0.3 $OD_{600\ nm}$ only. Examination of the current generated under external voltages of 75, 125, 175, 250 and 500 mV showed that the maximal current was 11, 23, 28, 54 and 94 mA/m^2, respectively. Cyclic voltammetry experiments demonstrated an anodic peak at 270 mV, which may imply oxidation of a vital molecule. The residual toluene concentration after 147 hour, in the BEC operated under external voltage was 22% on average, while in the control BEC it was 81% on average. The proteome analysis of bacterial cells grown in the BEC (125 mV) shows the appearance two groups of protein which are ascribed to charge transfer in the bacterial cells and from the cell to the electrode. The first group was composed of two proteins that play an important role in the respiratory electron transport chain: ubiquinol-cytochrome c reductase, iron-sulfur subunit and cytochrome c oxidase, cbb3-type. The second group was composed of three proteins that belong to a family of outer membrane proteins (Omp): OmpW, OmpF and Omp H1.

P27-17
Digestive M14 carboxypeptidases present in the midgut of insects

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Carboxypeptidases (CP) acting in digestive systems are metaloenzymes from family M14. Carboxypeptidases A (CPA) and B (CPB) hydrolyze substrates with hydrophobic or uncharged and basic residues, respectively. More recently a new enzyme was described; able to process peptides with an acid residue that was named CPGluMC. The enzyme specificity is determined by a residue located at the bottom of a substrate binding pocket. Material from midgut of *Tenebrio molitor* (Coleoptera), *Rhynchosciara americana* and *Musca domestica* (Diptera), and *Spodoptera frugiperda* and *Diatraea saccharalis* (Lepidoptera) were submitted to cDNA pyrosequencing and the obtained M14 enzyme sequences were analyzed. We considered only the contigs long enough to show the three residues that bind Zn^{++} plus the two catalytical residues, resulting in 39 sequences. *T. molitor* has two CP, one CPA and one CPB. The other insects have 8–10 CP summing up CPA, CPB and CPGluMC.The more abundant are CPAs, probably due to the higher diversity in non charged amino acid residues present in substrates. In *T.molitor, M.domestica and S.frugiperda*, where CP expression was analyzed along the midgut, all CP are found mainly in the posterior region of the midgut, in agreement with previous enzyme assays. In a cladogram, the CP branched according to the residue located at the bottom of the binding pocket. Carboxypeptidase gene duplication occurred before the evolutionary separation of the orders, resulting in two sequence branches (I and II). Further evolution took place after separation of the orders.

P27-18
Effects of boron on serum and liver macro elements in rabbits fed with high energy diet

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Objective: Investigate the effects of boron on serum and liver macro elements (total calcium, phosphorus, magnesium, sodium and potassium) in rabbits fed with high energy diet.

Methodology: 60 female New Zeland White rabbits, aged 8 months were involved in the study. Animals were divided into five groups, control 1 (alfalfa hay), control 2 (high energy diet containing 2800 kcal/kg metabolazable energy), remaining three groups were fed with high energy diet and boron was given by oral gavage at doses of 10 (exp 1), 30 (exp 2) and 50 (exp 3) mg/kg respectively in boron (B) compound (borax, $Na_2B_4O_7.10H_2O$). Levels of macro elements in serum, liver, alfalfa hay and high energy diet were determined by ICP-AES.

Results: Serum macro element concentrations did not change in control 1 and control two groups. Boron administration elevated serum total calcium levels in exp 1 (from 100.37 ± 4.38 to 220.10 ± 31.84 mg/l, $p < 0.01$), exp 2 (from 94.06 ± 3.89 to 146.87 ± 10.16 mg/l, $p < 0.001$) and exp 3 (from 103.41 ± 5.34 to 143.78 ± 8.30 mg/l, $p < 0.01$) compared to control 2 (from 104.56 ± 2.20 to 122.60 ± 14.22 mg/l, $p > 0.05$). Boron also elevated total phosphorus ($p < 0.05$), sodium ($p < 0.001$), and potassium ($p < 0.001$) concentrations at exp 2 and exp 3 groups compared to control 2 and control 1 groups ($p < 0.05$), whereas, it appeared to decrease Mg levels at the same groups, significantly ($p < 0.001$).

In control 2, total phosphorus levels were lower ($p < 0.01$) compared to control 1 in liver whereas the levels of total phosphorus were higher in the high-energy diet (4057.95 mg/kg) compared to alfalfa hay (3263.54 mg/kg). Calcium, potassium and magnesium levels were higher and sodium levels were lower in alfalfa hay compared to high energy diet. Boron administration did not present any significant changes on macro elements in liver.

Conclusions: Boron at 30 and 50 mg/kg doses elevated serum total calcium, phosphorus, sodium and potassium concentrations and decreased magnessium concentrations, however no change was detected in liver of rabbits fed with high energy diet. Because of the different levels, total phosphorus in alfalfa hay and high energy diet may be metabolised differently.

P27-19
Hydrogen metabolism in *Desulfovibrio gigas*

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Molecular hydrogen is the main component of diverse metabolic pathways, playing an active role in the metabolism of carbon compounds in bacteria. Several *Desulfovibrio* spp, *Desulfovibrio gigas* among them, have been proven to be able to use hydrogen as sole energy source due to the presence of an enzymatic group called hydrogenases. In *D. gigas*, two hydrogenases are described so far: one is the periplasmic hydrogenase belonging to the [NiFe] group (*HynAB*) and the second is a cytoplasmic membrane-bound hydrogenase (*ech*) previously described by our lab (1). As such, the hydrogen cycling model, originally proposed by Odom and Peck in 1981 (2) as a mechanism of energy conservation, could operate under lactate growth. Other models propose that hydrogenase activity could also be used simply as a way of regulating the cells redox state. As to better understand the role of the hydrogenases in the cell metabolism and the contribution of each of these enzymes, a mutant strain lacking the Ech-type cytoplasmic hydrogenase was constructed. The data obtained show reduced production of H_2 by the D*ech* mutant compared to the wild type strain under several growth conditions except during pyruvate fermentation, in which we observed the reverse. This result is surprising since the *ech* hydrogenase is expected to produce H_2. We are currently trying to understand the reason for this production. Also, we are continuing with the study of the *ech* and *hynAB* hydrogenases expression profiles at the levels of both mRNA and proteins. Concomitantly, a new mutant strain lacking the functional *hynAB* hydrogenase enzyme was made, in the same manner as the *ech* mutant, and its physiological profile was analyzed.

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P27-20
Form and function of membranes and their phospholipids of rat salivary glands: properties of membrane fusion between plasma membranes and isolated secretory granules, and consequent amylase release as saliva secretion

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The oral health has been gathering much attention because of not only periodontal diseases characterized by chronic inflammation but also the regulatory mechanisms for the controlling various body conditions. On the other hand, Salivary glands are playing an important role in the oral functions like digestion by the enzymes, mastication and swallowing by the mucus, resistance to infectious conditions including periodontitis and caries, and so on according to the saliva secretion. Salivary glands are continuously controlled by the autonomic nervous system and quickly respond to various agonists resulting in saliva secretion. This secretion, exocytosis, is phenomena of the membrane fusion between the plasma membranes and the secretory granular membranes and finally, the components of granule are secreted.

Recently, the molecular mechanisms of the exocytosis are focused to clarify the interactions and organizations of cells. The membrane physical properties play a very important role for the process of membrane fusion. We extensively investigated about the mechanisms of saliva secretion as a model cell for exocytosis. In order to clarify the characteristics of membranes which are not only from whole cells but also from secretory granule, their phospholipids and their fatty acids compositions were analyzed. Among the many steps of enzyme reaction for the biosynthesis of the major phospholipid, phosphatidylcholine, the reacylation enzyme activity is very important to estimate the phospholipid fatty acyl composition. Physicochemical properties, membrane fluidity, of various types of biomembrane isolated from the cells, and their phospholipid liposomes were measured using various types of spin probes. Under the reconstitution system for membrane fusion and amylase secretion consisted by isolated secretory granules and phospholipids liposomes or isolated plasma membranes, many modulation effectors in exocytosis are clarified.

P27-21
Hydroxytyrosol, a bio-active phenolic compounds of virgin extra olive oil, inhibits *in vitro, ex vivo* and *in vivo* angiogenesis

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Hydroxytyrosol is a phenolic compound abundant in virgin extra olive oil. Hydroxytyrosol has been shown to exhibit a number of

bio-active effects, including antioxidant activity, cardioprotective capacity, as well as antimicrobial, anti-diabetic, neuroprotective and anti-tumoral activities, among others. Based on the available scientific information, we established the following working hypothesis: 'Hydroxytyrosol could be an effective anti-angiogenic compound'. To test this hypothesis, we have performed an array of *in vitro* assays (all of them well established in our lab) carried out with cultures of endothelial cells. These assays allow for testing the effects these compounds on viability/survival/proliferation, migration, cell cycle and apoptosis of endothelial cells, as well as the potential of these cells to remodel extracellular matrix and to align in tubule-like structures on Matrigel. We have also tested the anti-angiogenic potential of hydroxytyrosol in the aortic ring *ex vivo* and the CAM (chorioallantoic membrane) *in vivo* assays. The present communication will show and discuss the results obtained, leading to the conclusion that hydroxytyrosol is, indeed, a new potent inhibitor of angiogenesis targeting key steps of the angiogenic process.

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P27-22
TRAP 5a interacts with cells of mesenchymal lineage and affects their differentiation

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Mesenchymal stem cell research has been crucial for the study of several pathological conditions such as obesity and in the field of regenerative medicine. Tartrate resistant acid phosphatase (TRAP), an enzyme traditionally used as a marker of osteoclasts, has recently been shown to be involved in the differentiation of mesenchymal stem cells into adipocytes. TRAP exists as two isoforms 5a and 5b, where 5a is mainly secreted while 5b is found intracellularly and has higher phosphatase activity. It has previously been shown that mice over-expressing TRAP 5a develop obesity but the molecular mechanisms behind this remain to be elucidated. The current project aims to shed light on the function of TRAP 5a in mesenchymal stem cells, and to identify interacting partners for TRAP on mesenchymal lineage cells. MSCs and pre-adipocytes, incubated with TRAP 5a at 4°C to prevent endocytosis, have been shown by immunocytochemistry to bind TRAP 5a to their cell surface suggesting the existence of a binding partner on the surface of these cell types. Moreover, differentiating pre-adipocytes treated with TRAP 5a show an increase in lipid droplet acquisition on early days of differentiation and also upregulate differentiation genes such as hormone sensitive lipase (HSL) and fatty acid binding protein 4 (FABP4) on days 1–5 of differentiation. Thus, the existence of an interacting partner for TRAP 5a eliciting a genomic response on mesenchymal lineage cells that promotes the differentiation is suggested.

P27m-23
Long-term cultures of macrophages show a similar behaviour as macrophages obtained from aged mice

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Cellular senescence is the process by which normal cells loose their ability to divide due to a permanent cell cycle arrest produced after continuous replication1. Senescent cells suffer a dysregulation of physiological functions such as an increased inflammatory background or an impaired antigen presentation. The accumulation of senescent cells seems to be involved in and is responsible for the generation of aging. Cellular senescence can be induced by two different pathways: telomere shortening and oxidative damage, although the nature and mechanism of this contribution remains largely unclear. In the present study, we have proved whether the functions of long-lasting cultures of macrophages from young mice resembles to the functions found in senescent cells responsible for the aging patterns described. For this purpose we have compared some crucial functions of macrophages (antigen presentation, pro-inflammatory cytokine expression and ROS production) and other well known pathways (DNA damage repair, STAT5 phosphorylation and oxidation) between bone-marrow derived macrophages from 6 to 8 weeks old Balb/c mice cultured *in vitro* for 7 (normal culture) and 21 (senescent culture) days. In conclusion we observed that long-term macrophage cultures resemble a similar behavior as cultures of macrophages obtained from aged mice, such as an impairment of the DNA damage repair and a reduction of GM-CSF induced pathways such as STAT5 phosphorylation or proliferation.

P27r-24
Ted1p monitors GPI lipid remodeling and coordinates this with the er exit of GPI-anchored proteins driven by the p24 complex

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The eukaryotic secretory pathway is responsible for delivery of a tremendous variety of proteins from the endoplasmic reticulum (ER) to their proper cellular location. However, the mechanisms by which the ER export machinery accommodates such diversity in secretory cargo remain poorly understood. Many cell surface proteins are posttranslationallly modified in the ER by their anchoring to the glycolipid glycosylphosphatidylinositol (GPI). We have recently shown that in yeast, the efficient ER exit of GPI-anchored proteins requires two consecutive processes: first, the structural remodeling of the lipid part on the GPI anchor leads to the concentration and sorting of GPI-anchored proteins to ER exit sites (ERES). Subsequently, the p24 protein complex recognizes correctly remodeled GPI-anchored proteins and links them to COPII components on these ERES, which ensures their efficient incorporation into COPII vesicles. In this study we show that GPI anchor recognition by the p24 complex occurs only after Ted1p has removed a phosphoresthanolamine group from the glycan backbone on the GPI anchor. Furthermore, we also show that Ted1p binds remodeled but not unremodeled GPI-anchored proteins. Therefore, our results strongly support a model in which Ted1p monitors GPI lipid remodeling and coor-

dinates this with the ER exit of GPI-anchored proteins driven by the p24 complex.

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P27r-25
The functional structure of er exit sites and early golgi requires cooperation between Erv14p and p24 complex cargo receptors

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ER transmembrane cargo receptors are major constituents of the early secretory pathway that continuously cycle between ER and Golgi apparatus. They function individually to mediate selective incorporation of specific cargo proteins into ER-derived vesicles by linking cargo in the luminal side and COPII coat assembly on the cytoplasmic side. This study reveals now that cargo receptors also act in concert with one another to cooperatively maintain the functional architecture of the early secretory pathway. We show that the cargo receptors Erv14p and p24 complex interact physically. The absence of both Erv14p and p24 complex disturbs the organization of both ER exit sites (ERES) and early Golgi, resulting in a severe defect in protein secretion and glycosilation. Interestingly, Grh1p and Bug1p, the yeast orthologues of mammalian tethering factors GRASP65 and GM130 respectively, that are normally associated to the transitional ER and the early Golgi, were completely redistributed to the cytosol in the double mutant strain. This suggests that cooperation between cargo receptors could be required for the recruitment of tethering factors to the ER exit sites. Surprisingly, the late Golgi was not affected by the double null mutations. These results support a cisternal maturation model in which the transitional ER matures into the early Golgi that finally fuses with a stable late Golgi compartment.

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P27-26
The AMPK activator metformin inhibits one of the main function of boar spermatozoa, motility

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AMP-activated kinase, AMPK, is a sensor that detects the cell energetic state and subsequently regulates metabolism. Our data show that AMPK protein is highly expressed in boar spermatozoa. Metformin (Metf) is a well-known activator of AMPK that is currently used as anti-diabetic drug. Metf studies have been focused in somatic cells and to date, there is not work performed in male germ cells, therefore, our aim is to study the effect of Metf in motility, one of the main spermatozoa functions. Motility was analyzed by ISAS® program after incubation of spermatozoa in presence or absence of Metf for different times (2, 24 hour) and concentrations (1–20 mM). Motility data reveals that as short as 2 hour treatment, Metf significantly reduces by 50% (5 mM) or by 100% (10–20 mM) the percentage of rapid spermatozoa (VAP > 80 μm/s), as well as significantly diminishes any sperm velocity: curvilinear VCL, straight VSL and the average VAP. Metf incubation for 24 hour causes greater effects in mentioned parameters and, in addition, leads to a significant reduction (63%, 5 mM) or blockade (100%, 10–20 mM) in the percentage of motile spermatozoa, as well as a significant decrease in the percentage of spermatozoa with progressive motility. Long Metf treatment causes a significant dose-dependent reduction of motility coefficients: linearity LIN, straightness STR, wobble WOB and beat cross frequency BCF. In summary, Metf treatment causes an inhibition of the motility in boar spermatozoa.

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P27-27
Hyaluronan modulates the migratory capability of human melanoma cell lines of different malignancy

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Hyaluronan (HA) is abundant in the extracellular matrix of normal skin tissues. This glycosaminoglycan actively contributes in metastasis formation and is known to modulate intracellular signalling pathways regulating proliferation and migration of malignant cells. Therefore we aimed to identify components of HA homeostasis (hyaluronan synthases (HAS) and HA-receptors CD44 and RHAMM) in human melanoma cell lines of different malignancy and to reveal its connection with the MAPK/ERK- and the calcineurin/PP2B (a Ser/Thr phosphoprotein phosphatase) pathways.

Non-metastasizing WM35 and aggressively metastasizing HT168 cell lines were used. Migration assays were performed in Boyden-chamber applying HA of two different molecular weights (300–800 and 1600 kDa) as chemoattractants. PP2B and ERK were inhibited with applications of Cyclosporin A (CsA) and PD098059 (PD), respectively.

We found that HT168 cells produced more HA, expressed higher level of HAS3 and RHAMM, migrated more intensely toward HA and were more sensitive to any pharmacological modulation than WM35 cells. Both cell lines expressed CD44 equally strong but we failed to detect expression of HAS1. Inhibition of ERK activity markedly elevated migration of both cell lines towards either HAs. Our data suggest that the molecular mass of the secreted HA and the expression pattern of HA-receptor may influence migratory properties of melanoma cells in a fashion dependent from the degree of malignity. PP2B and ERK1/2 both seemed to modulate HA-homeostasis of melanoma cells but acted oppositely in some aspects.

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P27-28
Detection of mobilizable and conjugative plasmids in biofilm-forming staphylococci isolated from clinical samples by using PCR and PFGE techniques

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Antibiotic use has been one of the greatest achievements of modern medicine. Nevertheless, in the last decades pathogenic bacteria resistant to antibiotics have become a global Health Problem. Two processes seem to behind the problem. First, conjugative plasmid transfer is the most efficient way of horizontal gene spread and it is considered as one of the major reasons for the emerging increase of multiple antibiotic-resistant bacteria. Second, bacterial biofilms are surface-attached microbial communities. The problems associated with biofilm infections in humans result from the distinct characteristics of biofilms, in particular their high level of resistance to antibiotics.

It has been demonstrated that conjugation occurs in biofilms and suggested that this connection may influence the chances of biofilm-related infection risks.

Therefore, it is necessary to study the molecular mechanism of conjugation in order to control the spread of antibiotic-resistance genes among bacteria. This approach involves the identification of conjugative plasmids that these bacteria contain and the biochemical and biophysical characterization of the proteins involved in the conjugative mechanism.

Coagulase-negative staphylococci are increasingly significant nosocomial pathogens, mainly as a consequence of their propensity to develop antimicrobial resistance. In this work fourteen different clinical biofilm-forming staphylococci are being studied by using PCR and PFGE techniques based on described conjugative plasmid homology (i.e., pSK41 and pT181). We are not only interested on identifying conjugative plasmids in these isolates, but also on elucidating the role of conjugative plasmids in the spread of antibiotic resistance in these biofilm-forming bacteria.

P27-29
Synthesis and antidiabetic activity of some novel chromonyl-thiazolidinediones/ rhodanines

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Diabetes mellitus (DM) is increasing in prevalence in the worldwide, a complex, chronic, and progressive disease, a condition characterized by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from the pancreatic beta cells (1). Thiazolidinedione compounds (glitazones) rank among the most used hypoglycemic agents in patients with type-2 diabetes. These drugs improve insulin responsiveness thereby increasing glucose transport, glucose oxidation and glycogen synthesis in the skeletal muscle. In addition, glitazones possess actions preserving pancreatic beta-cell function, regulate adipose tissue production of adipokines and contribute to the control of lipid storage (2).

Chromones are a group of naturally occurring compounds that are ubiquitous in nature especially in fruits, vegetables, nuts, seeds, flowers, and barks (3). Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diet of human (4). Molecules containing the chromone structure (for example chromone and flavonoids) have a wide range of biological activities. They are extremely safe and associated with low toxicity, making them excellent candidates for chemopreventive agents (5), and may exert a hypoglycemic effect by promoting peripheral utilization of glucose or enhancing the sensitivity of insulin in diabetic animals (6).

In this study, we report the synthesis and the *in vitro* insulin releasing activity of some novel compounds incorporating with two known bioactive heterocyclic nuclei such as substituted chromone and thiazolidinedione/rhodanine.

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P27-30
Is serum adiponectin level a marker for mortality in intensive care patients?

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Introduction: Adiponectin is a hormone secreted from adipose tissue and plays an important role in tissue inflammation, endothelial function, vascular reactivity and insulin sensitivity. These functions of adiponectin point out that its levels can be used to assess the severity of inflammatory response and organ dysfunction. Additionally, studies in animal models of sepsis showed that adiponectin modulates inflammation and survey. On the other hand, functions of adiponectin in intensive care patients have not been clarified yet.

In the present study, we investigated the relations of adiponectin levels with mortality occurring in one month and with the levels of inflammation markers CRP and procalcitonin in intensive care patients.

Material/Method: 25 patients (12 M–13 F, mean age 58.5 ± 17.9 years) who died (Ex group) and 63 patients (35 M–28 F, mean age 55.7 ± 18.9 years) who survived (S group) in one month after the sampling of blood were included in the study. Adiponectin levels were measured using eBioscience ELISA kit. CRP and procalcitonin levels were measured using turbidimetric and TRACE methods, respectively in Roche Cobas 8000 otoanalyzer and BRAHMS Kryptor compact analyzer.

Findings: Adiponectin levels did not differ significantly between Ex and S groups (8570 ± 1293 versus 8372 ± 1221 ng/ml, respectively, $p > 0.05$). On the other hand both procalcitonin (25.01 ± 11.54 versus 6.12 ± 2.10 ng/ml, $p = 0.019$) and CRP (17.13 ± 2.03 versus 11.96 ± 1.19 mg/dl, $p = 0.022$) levels were

significantly higher in Ex group compared to S group. Adiponectin levels correlated significantly with neither procalcitonin nor CRP levels.

Conclusions: In the present study we found that adiponectin levels were not related with mortality in one month in intensive care patients. It was suggested that further studies assessing mortality in longer terms (> 1 month) in higher number of patients are needed to clarify the relation of adiponectin levels with mortality in this setting.

P27-31
Role of GRK2 in developing vasculature and pathological angiogenesis

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G protein-coupled receptor kinases (GRKs) were initially identified as key players in the desensitization and internalization of multiple G protein-coupled receptors (GPCR). Besides such traditional role in GPCR signaling, recent data indicate that GRKs can also initiate alternative signalling pathways and participate in a variety of cellular processes. GRK2 can phosphorylate a growing number of non-GPCR substrates and associate with a variety of proteins related to signal transduction, thus displaying novel 'effector' functions that have been shown to underlie its participation in key cellular processes such as cell cycle, insulin resistance or cell migration. Consistent with such basic impact on cellular physiology, altered GRK2 expression has been linked to several cardiovascular disorders as well as to inflammatory and neoplastic conditions. Interestingly, we have found that embryonic lethal GRK2 knock-out mice display defects in embryonic angiogenesis and vasculogenesis consistent with an inefficient maturation of vessels, what suggest a critical role for GRK2 in vascular remodelling during development. By using primary cultured cells from wild-type and GRK2 hemizygous mice, we confirm that GRK2 modulates signaling, proliferation and migration of endothelial cells to different relevant physiological stimuli. Expression of GRK2 is also critical for *in vitro* tubule formation and for barrier function of the endothelium. Accordingly, *in vivo* neo-vascularization and vessel maturation was altered in both global and endothelium-specific GRK2 knockout mice. Finally, we have shown that endothelial expression of GRK2 is downregulated at both protein and mRNA level during tumour angiogenesis, but not in normal angiogenesis. Such downregulation leads to formation of more immature vessels, with a markedly defective investment of pericytes. The extent of neoplasic growth inversely correlates with the dosage of GRK2 in the endothelial vascular component. Overall, our results suggest that endothelial downregulation of GRK2 could play a relevant role in creating a permissive microenvironment for tumour progression.

P27-32
A_3-adenosine receptor effects on malignant melanoma cells

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Due to the limited response and poor prognosis associated with metastatic melanoma, new therapies are urgently needed. Tumour promoting activities have been associated with adenosine (ADO) (1). However, activation of A_3-adenosine receptor (A_3AR) induces inhibitory effects on melanoma cell growth (2).

We aim to evaluate proliferative and/or cytotoxic effects induced by A_3AR-activation on human metastatic melanoma cells. The proliferative/cytotoxic effects of ADO and/or adenosine agonists in human C32 melanoma cells, in the presence or absence of antagonist or nucleoside transporter inhibitors, were assessed using the MTT assay.

ADO (0.1–100 μM; 24 hour) promoted cell proliferation up to 117.90 ± 2.52 (n = 15; p < 0.001, from control cells), abolished by MRS 1220 (100 nM): 96.07 ± 2.40 (n = 3). Cl-IB-MECA showed a proliferative effect at lower concentrations: 100 nM (1 hour): 14.31% ± 2.04 (n = 18; p < 0.05); 10 nM (24 hour): 18.55 ± 5.08% (n = 18; p < 0.001), abolished by MRS 1220 (100 nM). Higher concentrations (30–100 μM) caused cytotoxicity (EC_{50} (1 hour) = 36 μM and EC_{50} (24 hour) = 70 μM), not abolished by MRS1220. In the presence of nucleoside transport inhibitors, the cytotoxic effect mediated by 50 μM of Cl-IB-MECA (−38.22 ± 2.48 n = 4; p < 0.001) was increased: NBTI (10 μM; -55.35 ± 2.13; n = 4; p < 0.001) or dipyridamole (10 μM; -71.15 ± 2.14; n = 4; p < 0.001).

Our findings suggest the proliferative effect elicited by exogenous ADO to be mediated by A_3AR. Activation of A_3AR by Cl-IB-MECA increases cell proliferation at nanomolar concentrations. At higher concentrations, Cl-IB-MECA causes cytotoxicity, not mediated by A_3AR activation. Endogenous ADO seems to protect human C32 melanoma cells from the cytotoxicity induced by high concentrations of Cl-IB-MECA.

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P27r-33
Cell responses to graphene oxide nanosheets

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Nanomedicine is focused in searching new nanosystems for biomedical application. Graphene is a single-atom-thick sheet of sp2-hybridized carbon atoms that has aroused great interest in the world of nanotechnology for its excellent properties. Similarly, graphene oxide (GO) is one of the most important graphene derivatives and has been extensively studied in recent years. Its biomedical application is being explored for drug delivery, bio-analysis and hyperthermia in cancer treatment. However, little is known concerning the GO biocompatibility and the cell response to this material. In order to evaluate the behaviour of different cell types in contact with GO nanosheets, human osteosarcoma Saos-2 cells as tumour cell line, murine MC3-T3 preosteoblasts as undifferentiated osteoblast-like cells and murine Raw 264.7 macrophages were cultured in the presence of GO with 1 (1-GOs) and 6 arms (6-GOs) of polyethylene glycol (PEG). The incorporation of both GOs by each cell type does not induce plasma membrane damage but it produces a delay in cell proliferation affecting the cell cycle phases and increasing the content of intracellular reactive oxygen species. However, no morphological alterations are observed. A decrease of proinflammatory cyto-

kines production in human Saos-2 cells is also detected suggesting the absence of an inflammatory response to this material.

P27-34
A role for the PKCeta ATP binding site polymorphism (374I) in kinase activity and secretion of inflamtory mediators relevent to stroke and rheumatoid arthritis

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In a search for genes increasing the susceptibility for stroke and rheumatoid arthritis (RA) in the Japanese population the PKCeta gene, located on chromosome 14, was identified. In particular a SNP (*V374I*) at theATP-binding site was in correlation with this increased susptebility. PKCeta is a member of PKC novel subfamily, expressed predominantly in epithelia and in cells undergoing high turnover. It was shown to play a role in cell cycle regulation, differentiation, proliferation and anti-apoptotic processes. In order to assess the cellular effects of this polymorphism we generated the valine (V) to isoleucine (I) substitution and examined its effects on PKCeta kinase activity, cellular localization and the secretion of cytokines: nitric oxide (NO), Interlukin-6 (IL-6) and tumor narcosis factor alpha (TNF alpha). Our results demonstrated that PKCeta-*374I* is more active as a kinase, showing increased autophosphorylation and phosphorylation on the external substrates MBP and MARCKS. However this increased kinase activity did not affect cellular localization since both PKCeta forms were localized in the Golgi, ER and the cytoplasm and translocated to the nuclear envelope (NE) and cell membrane upon PMA activation. Our results show an effect of PKCeta and 374I on cytokine secretion. Our results showed that LPS induced NO levels were elevated in MCF7 cells expressing PKCeta-*374I* compared to PKCeta-*374V*. Furthermore, TNF alpha and IL-6 secretion was also increased by PKCeta following LPS treatment in MCF7 cells. The elevation in IL-6 and TNF alpha levels with both PKCeta forms suggests that it has a role in inflammatory related processes. Our studies demonstrate a role for PKCeta *V374I* substitution increasing IL-6, TNF alpha and NO levels could suggest a connection between PKCeta and secretion to the increased susceptibility to RA and stroke.

P27r-35
p73 function regulates sprouting and network formation during endothelial differentiation of murine embryonic stem cells (mESC)

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Angiogenesis, defined as the formation of blood vessels from pre-existing ones, plays a central role during physiological processes such as embryogenesis, as well as during some pathological conditions like tumor progression and metastasis. Angiogenesis involves a coordinated control of endothelial cell behaviour at levels of cell migration, proliferation, differentiation and cell-cell communication. This behaviour is regulated by fine-tuned signalling pathways, both at transcriptional and post-transcriptional levels. Therefore, to decipher the molecular mechanisms controlling all these processes can be of great interest not only for basic research but also for therapy.

The p53 gene family is constituted by the transcription factors p53, p73 and p63. There is evidence that suggests that p73 maintains some unique functions not shared with p53, like the p73-specific role in cellular differentiation and development. Involvement of p73 in angiogenesis has been addressed from different views and the results are to some extent controversial.

As a first approach to elucidate a possible role of p73 in angiogenesis we address a possible function of p73 in endothelial differentiation. For this purpose we have generated mouse embryonic stem (mES) E14Tg2α cell lines which express p73-dominant negative proteins: E14Tg2α-DDp73. These stable cell lines have been used to evaluate the effects of the functional inhibition of p73 in an *in vitro* model based on the formation of embryoid bodies (EBs), spheroids of differentiating mES in either a bidimensional or a tridimensional environment. In this model, the functional inhibition of p73 seemed to impair sprouting and network formation during endothelial differentiation. Future experiments will help us to clarify the possible role of p73 in angiogenesis.

P27-36
p73 regulates mouse neural progenitors self-renewal and the organization and architecture of the neurogenic niches

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The question of how neural progenitor cells maintain its self-renewal throughout life is a fundamental problem in cell biology with implications in cancer, aging and neurodegenerative diseases. In this work, we have analyzed the p73 function in embryonic neural progenitor cell biology using the neurosphere (NS)-assay and showed that p73-loss has a significant role in the maintenance of neurosphere-forming cells in the embryonic brain. A comparative study of NS from $Trp73^{-/-}$, p53KO, p53KO;$Trp73^{-/-}$ and their wild-type counterparts demonstrated that p73 deficiency results in two independent, but related, phenotypes: a smaller NS size (related to the proliferation and survival of the neural-progenitors) and a decreased capacity to form NS (self-renewal). The former seems to be the result of p53 compensatory activity, whereas the latter is p53 independent. We also demonstrate that p73 deficiency increases the population of neuronal progenitors ready to differentiate into neurons at the expense of depleting the pool of undifferentiated neurosphere-forming cells. Analysis of the neurogenic niches demonstrated that p73-loss depletes the number of neural-progenitor cells, rendering deficient niches in the adult mice with an abnormal architecture.

Altogether, our study identifies TP73 as a positive regulator of self-renewal with a role in the maintenance of the neurogenic capacity and in the architecture of neurogenic niches. Thus, proposing p73 as an important player in the development of neurodegenerative diseases and a potential therapeutic target.

P27-37
Novel MUC1 aptamer selectively delivers cytotoxic agent to cancer cells *in vitro*

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Chemotherapy is a primary treatment for cancer, but its efficacy is often limited by the adverse effects of cytotoxic agents. Targeted drug delivery may reduce the non-specific toxicity of chemotherapy by selectively directing anticancer drugs to tumor cells. MUC1 protein is an attractive target for tumor-specific drug delivery owning to its overexpression in most adenocarcinomas. In this study, a novel MUC1 aptamer is exploited as the targeting ligand for carrying doxorubicin (Dox) to cancer cells. We developed an 86-base DNA aptamer (MA3) that bound to a peptide epitope of MUC1 with a K_d of 38.3 nM and minimal cross reactivity to albumin. Using A549 lung cancer and MCF-7 breast cancer cells as MUC1-expressing models, MA3 was found to preferentially bind to MUC1-positive but not MUC1-negative cells. An aptamer-doxorubicin complex (Apt-Dox) was formulated by intercalating doxorubicin into the DNA structure of MA3. Apt-Dox was found capable of carrying doxorubicin into MUC1-positive tumor cells, while significantly reducing the drug intake by MUC1-negative cells. Moreover, Apt-Dox retained the efficacy of doxorubicin against MUC1-positive tumor cells, but lowered the toxicity to MUC1-negative cells ($p < 0.01$). The results suggest that the MUC1 aptamer may have potential utility as a targeting ligand for selective delivery of cytotoxic agent to MUC1-expressing tumors.

Keywords: MUC1, aptamer, targeted drug delivery, doxorubicin.

P27r-38
Is TRIM32 a transcriptional target of p73?

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The p53-gene family is constituted by the transcription factors p53, p73 and p63. These genes play key roles as regulators of proliferation, differentiation, cell death, stem cell self-renewal, and cell fate commitment. The TP73 gene exhibits a dual nature which resides in the existence of TA and DNp73 variants. The TA-proteins are transactivation competent, while, the DN-isoforms lack the transactivation domain and can act as dominant-negative repressors of p53 and TAp73. Recently, our group have demonstrated that p73, is deeply involved in maintenance of neural stem/progenitor cell (NPC) self-renewal. p73 is a positive regulator of self-renewal by maintaining the undifferentiated phenotype of the NPCs in a p53 independent manner.

In neurospheres cultures from p73 deficient embryos, impaired neurosphere formation correlated with an increased number of asymmetric divisions in those cultures. However, there is no data on the possible role of p73 in the regulation of asymmetrical divisions of stem cells. In this context, the neuronal fate determinant TRIM32 has been shown to segregate asymmetrically in mouse neural progenitor cells that are differentiating into neurons during mouse embryonic brain development and to regulate, in this way, NPC self-renewal. Hence, we hypothesized the existence of a functional link between p73 and TRIM32. How the expression of TRIM32 is regulated is currently entirely unclear. Nevertheless, genome-wide analysis of gene expression through microarray hybridization in K562 cells that overexpressed DNp73, revealed a down-regulation of TRIM32 in these cells suggesting that TRIM32 could be a p73 transcriptional target (Marques-Garcia *et al.,* 2009). Reinforcing this idea, in silico prediction of p53-responsive elements within the human TRIM32 locus using p53 Family-Target Genes database, unveiled several p53-binding sites within the TRIM32 promoter.

To address this question we have analyzed the transcriptional regulation of TRIM32 by the p73 transcription factor, and demonstrated that TRIM32 is a direct target of TAp73.

P27-39
Generation and study of a bimodal lentiviral vectors for the transcriptional analysis of the TP73 gene

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Multicellular organisms have developed the ability to regenerate many of their tissues to maintain the homeostasis. The ability of certain adult tissues to regenerate throughout life is a direct result of stem cell function. A stem cell is a cell that has the ability to divide for indefinite periods giving rise to identical stem cells (self-renewal) and, given the right signals, different into cell types that make up the organism (pluri- or multi- potency). Therefore, in these cells the mechanisms that regulate genetic stability, proliferation, apoptosis and differentiation must be tightly regulated in order to maintain a controlled self-renewal and multipotency. In somatic cells, the members of the p53 family are deeply involved in the regulation of such processes.

There is evidence that suggest that p73 maintains some unique functions not shared with p53, like the p73-specific role in cellular differentiation and development. Our working hypothesis is that p73 may be playing a significant role in the regulation of self-renewal and differentiation of stem cells. To address this possibility we have generated a bimodal reporter system that allows us to analyze the TP73 transcriptional activity which it would be a useful tool with a potential biotechnological interest.

P27r-40
The obestatin/gpr39 system is up-regulated by muscle injury and functions as an autocrine regenerative system

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Adult skeletal muscle has a remarkable capacity regenerative potential, which is attributable to an elaborate interaction

between extrinsic and intrinsic regulatory signals that regulate myogenic process. In the present work, we showed that obestatin, a 23-amino-acid peptide encoded by the ghrelin gene, and the GPR39 receptor, are expressed in rat skeletal muscle being up-regulated upon experimental injury. To delineate the role in muscle regeneration, L6E9 cells were used for *in vitro* assays. For *in vivo* assays, skeletal muscle tissue was obtained from male rats under continuous subcutaneous infusion of obestatin. In differentiating L6E9 cells, preproghrelin expression, and thus obestatin, increased during myogenesis being sustained throughout terminal differentiation. Autocrine action was demonstrated by neutralization of endogenous obestatin secreted by differentiating L6E9 cells using specific anti-obestatin antibody. Knockdown experiments by preproghrelin siRNA supported that obestatin contributes to myogenic program. Furthermore, GPR39 siRNA reduced obestatin action and the myogenic differentiation. Obestatin treatment showed to regulate myoblast migration and proliferation. Remarkably, obestatin stimulation increased myogenic differentiation of L6E9 cells. The relevance of obestatin actions was confirmed *in vivo* by up-regulation of Pax-7, MyoD, Myf5, Myf6, myogenin and myosin heavy chain (MHC) in obestatin-infused rats compared to saline-infused rats. These data delineate a novel mechanism whereby the obestatin/GPR39 system is coordinately regulated as part of the myogenic program and operate as an autocrine signal regulating skeletal myogenesis.

P27r-41
The rab gtpase ypt1p and the p24 transmembrane protein complex cooperate in vesicular transport within the early secretory pathway

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Vesicular transport through the eukaryotic secretory pathway is essential for cellular function and multicellular development. This evolutionary conserved process depends on cytosolic coat protein complexes that form vesicles, select specific cargo molecules for incorporation into the vesicles and direct the vesicles to their correct intracellular destination. Two types of coats operate in the early secretory pathway: COPII mediates the export from the endoplasmic reticulum (ER), while COPI is involved in the retrograde transport from the Golgi to the ER and between Golgi cisternae. During the last years we have obtained evidence in yeast indicating that a conserved group of integral membrane proteins, referred to as the p24 family, regulate multiple vesicular trafficking events by controlling COPII and COPI membrane recruitment. p24 proteins, which contain high-affinity COPII and COPI binding signals, are assembled into heteromeric complexes that continuously cycle between ER and Golgi. To gain more insights into the functions of the yeast p24 complex in the early secretory pathway, we performed a screen for mutations that induce synthetic enhancement upon disruption of the *EMP24* gene. We identified a strong genetic interaction between *EMP24* and *YPT1*, an essential gene encoding a small GTPase of the Rab family required for multiple vesicle budding and tethering events at the ER-Golgi shuttle. The phenotypic characterization of the double mutant *emp24*Δ *ypt1-3* suggests that the cycling p24 complex cooperates with Ypt1p in anterograde COPII and retrograde COPI vesicular transport within the early secretory pathway.

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P27-42
MUC1 mucin and steroid hormone receptors in bovine endometrium (BEEC) and oviduct (BOEC) epithelial cells in culture

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Progesterone-dependent regulation of anti-adhesion MUC1 mucin appears to be an important factor in determining endometrial receptivity. MUC1 plays a role in protecting the endometrium from microbial attack, but must be lost in order for embryo implantation to occur. This is facilitated in some species by progesterone down-regulation of endometrial MUC1 to coincide with the implantation phase of the reproductive cycle. The mechanism of this type of regulation remains to be established, but stimulation of human *MUC1* promoter by progesterone, mediated by its receptor, has been shown. Our main goal is to study the unknown transcriptional regulation of *MUC1* in bovine endometrium and oviduct epithelial cells, specifically investigating how it is influenced by treatments with progesterone and oestrogen. To this end, we have established primary cultures of bovine endometrium (BEEC) and oviduct (BOEC) epithelial cells using bovine uteri and oviducts at days 1–3 of the oestrous cycle, accurately determined by the presence of a *corpus hemorrhagicum*, and they have been characterized for the expression of oestrogen/progesterone receptors and *MUC1* by real-time RT-PCR and Western blotting. BEEC had lower levels of transcription for all the genes analyzed and lower levels of MUC1 protein, when compared with BOEC. Knowledge of how *MUC1* expression can be regulated in uterine epithelium may aid assisted reproduction technologies, by decreasing MUC1 and increasing the availability of the uterine cell surface to the embryo, thereby improving pregnancy rates and reproductive efficiency, in domestic animals as well as in humans.

P27-43
Galanin receptor 3 is mediating important functions in polymorphonuclear neutrophils

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Galanin is a bioactive neuropeptide that participates in the recruitment and activation of polymorphonuclear neutrophils (PMNs). To date, 3 galanin receptors (GALR1, GALR2, GALR3) belonging to the G-protein coupled receptor family are known, however, the receptor(s) involved in PMN processes are unclear. Consequently, we aimed to determine GALRs messenger RNA (mRNA) expression in PMNs, and ascertain if these receptors are involved in PMN functions.

Using reverse transcription polymerase chain reaction (RT-PCR) we were able to show that GALR2/3 receptors are found to be expressed in human resting PMNs. Additionally, immunoblotting using specific GALR2/3 antibodies confirmed that mRNAs detected by RT-PCR were translated into proteins. To further investigate the receptor subtype mediating galanin PMN functions, we were using the specific GALR3 antagonist SNAP-

37889. We found that SNAP-37889 treatment significantly reduced the degranulation of azurophilic/primary and secretory granules in peptide stimulated PMNs. Furthermore, a decrease of galanin mediated beta-2-integrin CD11 expression was observed.

Additionally, SNAP was also able to block the galanin induced increase of dynamic mass redistribution (DMR) within the cell, which was measured by Enspire cell based labelfree technology. *In vivo*, we showed that galanin mediated PMN recruitment, rolling and adhesion to the blood vessel wall in rat inflamed knee joints is GALR3 dependent.

Taken together, we could demonstrate that GALR3 is mandatory for galanin-reliant functions in PMNs and that SNAP-37889 provides a valuable tool for elucidating pharmacological effects of GALR3 antagonism in inflammatory disorders.

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P27-44
Assessment of possible cytotoxic effects of ciprofloxacin, oxociprofloxacin, zinc oxide nanoparticles and melamine to vero cells and protective effects of vitamin E and coenzyme Q10

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The purpose of this study was to evaluate the possible time- and dose-dependent cytotoxic effects of ciprofloxacin (CPFX), oxociprofloxacin, zinc oxide (ZnO) nanoparticles and melamine on Vero cells. The cultured cells were exposed to various concentrations of CPFX (0.5–300 mg/l, for 24, 48, 72 and 96 hour), oxociprofloxacin (1–30 mg/l, for 24, 48, 72 hour), zinc oxide nanoparticles (0.1–800 mg/l, for 24, 48, 72 hour), or melamine (2.5–500 μM, for 24, 48, 72 and 96 hour) for different incubation periods as indicated in the parenthesis, and cytotoxicity was determined by MTT assay. Cytotoxicity profiles of four xenobiotics in Vero cells were as follows: Following 24 and 72 hour of incubation, a slight cell proliferation was determined at 0.5 ($p < 0.5$) and 5 mg/l concentrations of CPFX, respectively. A gradual decrease was noted in viability of cells ≥50 mg/l of CPFX following 24, 48 and 72 hour of incubations and ≥0.5 mg/l concentrations of the drug at 96 hour. As incubation period was longer, decrease in cell proliferation was more pronounced. Cytotoxicity profile of oxociprofloxacin, a metabolite of CPFX, was found slightly different from the parent drug: ≥10 mg/ml of oxociprofloxacin for 24 and 48 hour, and >2 mg/ml oxociprofloxacin for 72 hour caused marked decreases in cell proliferation. With oxociprofloxacin, an increase in cell proliferation was noted only at 2 mg/ml for 24 hour, and 1 mg/ml at 72 hour of incubation. However, neither ZnO nanoparticles nor melamine were found cytotoxic in the present model system, and only slight decreases were observed in cell survival with melamine. Pretreatment of cells with vitamin E (100 μM) or coenzyme Q_{10}(30 μM) for 4 hour provided complete protection against CPFX-induced cytotoxicity. Data obtained in this study suggest that cytotoxicity of CPFX might be related to oxidative stress and confirm our previous results obtained with CPFX in diverse systems.

P27-45
Detection method for identification of *Pueraria mirifica* in processed foods

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In this study, it was selected ribulose bisphosphate carboxylase(*rbcL*), RNApolymeraseC(*rpoC1*), intergenic spacer(*psbA-trn*H), and second internal transcribed spacer(*ITS2*) used an identification of plant species for discrimination of *P. mirifica* in foods. For sequencing, we obtained 719, 520, 348, and 507 bp amplicon each using universal primers from selected genes of *P. mirifica*. The sequence of *rbcL, rpoC1*, and *psbA-trnH* had difficult to design primers because of high homology about *P. mirifica* and related species. But, incase of *ITS2* had a differentia designed four pairs of oligonucleotide primers. The result of PCR using designed primers about *P. mirifica, P. lobata*, and *B. superba* was not produced non-specific bands from SFI12-miri-6F/SFI12-miri-7R and SFI12-miri-6F/SFI12-miri-8R primer, and the expected sizes were 137bp and 216bp in *P. mirifica* each. Therefore, the species-specific primers distinguished *P. mirifica* were able to apply food materials and processed foods, so this specific PCR method would be applicable to managea food safety for illegally distributed products in markets and internet shopping malls.

P27-46
Effect of hypoxia on lung gene expression and proteomic profile: insights into hypoxia response and pulmonary surfactant

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Pulmonary surfactant, a lipid-protein complex covering the air-liquid interface of alveoli, is essential for stabilizing the lung, preventing alveolar collapse at the end of expiration. To do so, surfactant reduces surface tension by forming an interfacial film, which has to be crossed by oxygen to reach the pulmonary epithelium and the capillary. The effect of the presence of the pulmonary surfactant layer on oxygen diffusion has not been properly evaluated nor whether adaptation of the lung to hypoxia (chronic or acute) could also involve compositional and/or structural changes in surfactant. On the other hand, type II pneumocytes -the cells in charge of synthetising and secreting surfactant- have been shown recently to produce haemoglobin, suggesting a potential connection of pulmonary surfactant with oxygen availability at the respiratory interface. In the present work, we have studied the effect of exposure to hypoxic conditions on the composition and function of pulmonary surfactant. The expression of proteins related to hypoxia (HIF-1α, HIMF, α-globin, β-globin) and to surfactant (SP-A, SP-B, SP-C, SP-D, ABCA3) has been also examined at the mRNA level in lung tissue from rats exposed to normoxia or to 10% oxygen during 72 hours. The protein content was also analyzed in bronchoalveolar lavage, as well as the surface activity of surfactant, as assessed by spreading assays in surface balances. Changes in the

proteomic profile of lung tissue and bronchoalveolar lavage from hypoxic with respect to normoxic rats were analyzed by 2D-DIGE (Two-Dimensional Difference Gel Electrophoresis). The results showed no changes in the mRNA levels of surfactant proteins, whereas we observed a significant increase in the levels of alpha and beta globins, indicating that haemoglobin could be involved in the response of lung tissue to hypoxia. On the other hand, the analysis of the proteomic profile revealed a complex response, including up and downregulation of several proteins involved mainly in immune response and inflammation, as well as in redox balance, whose relevance will be discussed.

P27-47
Evaluation of pH effects on the growth of *Enterococcus faecalis* DISAV1022 and its production of biogenic amines

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Enterococcus faecalis DISAV1022 was isolated from a sample of Robiola of Roccaverano, a typical Piedmont cheese. Previous studies highlighted its ability to produce two biogenic amines: tyramine and β-phenylethilamine, the decarboxylation products of tyrosine and phenylalanine respectively (1). The kinetic of the production of these amines in physiological conditions seem to suggest that a unique enzyme, named tyrosine decarboxylase, is able to decarboxylate both the amino acids with a higher affinity for tyrosine (1). In this study the effect of the pH on the production of biogenic amines by *E. faecalis* DISAV1022 was analyzed; it is interesting to evaluate this effect considering that one of the main function of bacterial amino acid decarboxylation is the alkalinization of the environmental pH. To gain this goal the strain was grown in a CDM medium fortified with 2 g/l of tyrosine and phenylalanine in a 5 l fermentor performing inocula from pre-cultures in order to have an initial $OD_{600\ nm}$ of 0.1. The fermentations were performed at free pH (initial medium pH 6.5), pH 4, 5, 6, 7 and 8. *E. faecalis* DISAV1022 revealed to have an optimum pH growth values of 7 as suggested by a final $OD_{600\ nm}$ of around 8.5; it displayed a good growth rate also at pH 8 at which its final $OD_{600\ nm}$ is around 7. On the contrary the final OD values were lower at pH 6 and free pH (around 4.5) and the growth resulted to be strongly inhibited at pH 5 as suggested by a 24 hours lag phase and by a final $OD_{600\ nm}$ around 2.5. Finally at pH 4 the strain was not able to grow at all. As regards the production of biogenic amines the first HPLC experiments, performed at pH 5, 6 and free pH, indicate that tyramine production is not influenced by pH: in fact the whole supplemented tyrosine is converted into tyramine during the exponential growth phase. On the contrary the production of β-phenylethylamine started when tyrosine is fully depleted, but it significantly increased at higher pH values. These preliminary observations can suggest two different hypothesis: the affinity of tyrosine decarboxylase for phenylalanine increases at higher pH values or a second enzyme, a phenylalanine decarboxylase, becomes active at higher pH values.

Reference

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P27-48
Investigation of the antimicrobial effect of an alkaloid from *Papaver rhoeas* K

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The growing threat of antimicrobial resistance is urging scientists to seek for antimicrobial agents in alternative sources. In this respect, due to their chemical diversity plants serve as invaluable sources for therapeutic agents for new drug leads. Alkaloids primarily synthesized by plants are secondary metabolites with potential antimicrobial activities against some bacteria and fungi. In this work, the antimicrobial effect of (−)-roemerine of *Papaver rhoeas* K. on *Escherichia coli* TB1 cells has been investigated in detail. This alkaloid has been found to possess antimicrobial activity on a number of microorganisms. Moreover it is not cytotoxic to human epithelial and monkey kidney cell-lines. Therefore (−)-roemerine can serve as a potential drug candidate. In order to investigate the effect of the alkaloid on *E. coli* TB1, cells were grown in its presence and absence and the change in growth profiles were compared. (−)-Roemerine was dissolved in dimethylsulfoxide (DMSO) and added to actively growing cells as optical density of the cells reached at 0.5 at 600 nm and change in optical density of the cells at 600 nm was monitored. The final concentration of the alkaloids in the media was adjusted to 25, 50, or 100 μg/ml. Control cultures were treated with DMSO without the alkaloid. Growth profiles showed that the presence of 100 μg/ml (−)-roemerine caused cell growth to cease with is clearly related to the disruption of the intricate network of cellular processes.

P27-49
Circulating microrna expression profiling in healty subjects in the east mediterranean of Turkey

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression at the posttranscriptional level by either degrading or blocking translation of messenger RNA targets. They have roles in growth, differentiation, proliferation and cell death by suppressing one or more target genes. miRNAs may be located in the introns and exons of protein-coding genes or in intergenic regions. More than 50% of miRNAs are found in cancer associated regions of the genome or in fragile sites; this suggests that miRNAs have important roles in the pathogenesis of neoplasias. The aim of this study was to investigate the profiling of circulating miRNAs expression in volunteer healty subjects. We evaluated the circulating miRNAs expression in three hundred and two healty subjects from ın the East Mediterranean of Turkey. EDTA-anticoagulated peripheral blood samples were centrifuged at 4000 rpm for 15 min then the plasma was separated. cDNA's were obtained from the isolated plasma miRNAs and 768 different miRNAs were analyzed with High Throughput Real-Time PCR (qRT-PCR) device (Fluidigm, Biomark, USA) by using 96.96 Dynamic Array IFCs. From the 768 miRNA analyses, expressions of *let-7g, miR-30d, miR-378, miR-18a, miR-*

186,miR-1291, miR-223, miR-10a, miR-10b, miR-1180, miR-1183, miR-1255B, miR-1274A, miR-1274B, miR-1290, miR-135b, miR-136, miR-141, miR-147, miR-148b, miR-150, miR-151-3p, miR-151-5P, miR-152, miR-154, miR-181a-2, miR-17, miR-183, miR-184, miR-206, miR-22, miR-208, miR-212, miR-190, miR-204, miR-221, miR-197, miR-191, miR-195, miR-193a-5p, miR-205, miR-203, miR-193b, miR-199a-3p, miR-218, miR-210, miR-27a, miR-34a, miR-34b, miR-25, miR-302a, miR-23a, miR-320B, miR-324-5p, miR-432, miR-497, miR-519b-3p, miR-424, miR-425, miR-543, miR-409-3p, miR-519e, miR-548b-5p, miR-532-3p, miR-548a, miR-532, miR-510, miR-422a, miR-484, miR-491-5p, miR-494, miR-409-5p, miR-423-5p, miR-505, miR-433, miR-518d, miR-508, miR-502-3p, miR-411, miR-515-3p, miR-483-5p, miR-520a, miR-520d-5p, miR-431, miR-451, miR-490, miR-624, miR-601, miR-599, miR-551b, miR-603,miR-645, miR-648, miR-598, miR-642, miR-652, miR-570, miR-548c, miR-660, miR-628-5p, miR-574-3p, miR-590-5p, miR-654-3p, miR-576-3p, miR-597, miR-548c-5p, miR-548d, miR-579, miR-9, miR-93, miR-766, miR-720, miR-99b, miR-942, miR-7, miR-875-3p, miR-885-5p, miR-744, miR-886-5p were detected in healty subjects. As a result, 139 miRNA expressed while 517 was not expressed from 768 different miRNAs in healty subjects.

P27-50
The behaviour and angiogenic properties of human endothelial progenitor cells under hypoxic conditions

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Endothelial progenitor cells (EPC) could represent therapeutic tools for restoring the ischemic heart function, by either direct or paracrine effects. However, after homing into infarcted myocardium, the behavior of EPC might be modified by the hypoxic conditions. This study aimed to evaluate the behaviour and angiogenic properties of EPC in hypoxic conditions. EPC were obtained from peripheral blood of healthy volunteers by 7-day culture of mononuclear cells in MV2 medium. After hypoxia treatment (1% O_2, 24 hours), the conditioned medium (EPC-CM) was used for determination of angiogenic properties and the cells were evaluated by Western-blot and RT-PCR analysis. EPC expressed both the markers of the monocyte-macrophage and of endothelial cell (EC) lineages and had the specific pattern of 'early outgrowth EPC' described before. Hypoxia did not significantly modify the viability of EPC, induced an increase in secreted VEGF but not of SDF level. Compared to mature EC, EPC secreted larger amounts of SDF in both normal and hypoxic conditions. The angiogenic properties were similar in hypoxic and normal conditions. However, cell index determined with xCELLigence system revealed that EPC-CM supported the proliferation, but not the adhesion, of EC *in vitro*. The results suggested that soluble factors secreted by EPC might not be able to support the engraftment of EC at the site of angiogenesis. However, this shortage was compensated by mesenchymal stem cells (MSC)-CM added to the culture medium of EC. In conclusion, hypoxia does not modify the behaviour and angiogenic properties of EPC. Soluble factors secreted by EPC and MSC can be combined to promote the EC adhesion, which along with chemotaxis and proliferation are required for efficient angiogenesis.

P27-51
Cyanine bases as optical sensors for the specific recognition of cancer markers

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Recently, a slight decline in the incidence of cancer has been achieved worldwide, but still long-term mortality rates remain high. For successful therapy, early diagnosis of cancer plays the key role. Implementation of early detection in traditionally used clinic methods is necessary for significant reduction of the morbidity and mortality caused by cancer. This recognition can be based on a specific interaction of diagnostic agents with suitable molecular partners; i.e. cancer biomarker, represent molecular signatures of cell phenotype. As consistent with chemical structure, the cancer marker can be classified as saccharides, steroids, lipid derivates and others.

Their structure analysis indicated that some cancer saccharide markers (anionic polysaccharides), lipid derivates (cardiolipin) and cancer steroid markers (sulphate steroids) can be recognised by same type of agent (cyanine bases). Our results showed that these agents showed high affinity and selectivity for target analytes coupled important spectral changes, which were specific for the type of cancer markers.

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P27-52
Recognition of arginine/serine repeats by SR protein kinase 1: insights from molecular dynamics simulations

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Arginine/Serine (RS) repeats are found in several proteins in metazoans with a wide variety of functions, many of which are regulated by SR protein kinase 1 (SRPK1) mediated phosphorylation. However, their conformational preferences and the recognition mechanism used by SRPK1, remain rather elusive.

Using molecular dynamics (MD) simulations, we recently showed that, unphosphorylated peptides containing short repeats of consecutive RS dipeptides adopt transient α-turn-like conformations, whereas Ser-phosphorylation induces more compact structures, irrespective of the repeat length, probably serving in recognition by basic partners.

Here, we present MD simulations of a conserved peptide (R2') of lamin B receptor (LBR), overlapping with its RS repeats, both in isolation and in complex with SRPK1. The program GROMACS4, through an updated version of the Gromita GUI recently developed in our lab and the Amber99SB-ILDN force field, which we showed that is able to produce reliable MD results, were used for this purpose.

Our MD results revealed that, the RS portion of R2' also adopts transient α-like conformations in its free form, whereas it

docks in an extended conformation into the same, distant from the active site, acidic docking groove of SRPK1 used for much longer substrates. In combination with literature and biochemical data, these findings show that, the C-terminal part of the LBR RS domain constitutes a recognition platform for SRPK1. In addition, our MD data suggest that, docking to SRPK1 may promote unfolding of the RS repeats destined to be phosphorylated.

In total, our studies on the RS domain of LBR, shed light on the conformational preferences of the RS repeats as well as on aspects of their recognition by SRPK1 and support the idea that, the RS repeats share a common recognition mechanism by SRPK1, irrespective of their length, thus adding to knowledge towards a full understanding of their phosphorylation mechanism.

P27r-53
Salivary surrogates of markers of muscle injury and training load

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This study examined the variation in salivary nitric oxide (NO), alpha-amylase (sAA), total protein (sPT) and serum markers of muscle injury and exercise capacity during 21 weeks of training in elite swimmers. Samples of saliva and blood were collected once a month during 5 months from eleven male professional athletes during their regular training season. The variation in each marker throughout the 21 weeks was compared with the dynamics of training volume, intensity and load. Unstimulated whole saliva was assessed for NO, sAA and sPT whereas venous blood was assessed for lactate dehydrogenase, creatine kinase, cortisol, catecholamines as well as NO. We observed strong correlations between salivary and plasma NO as well as sAA and sPT with catecholamines. Also, similar variations in the concentration of NO and the markers of muscle injury were found. Finally, the salivary constituents showed proportional response to training intensity and load. These findings provide appealing evidence for the utilization of salivary constituents in sports medicine to monitor training programmes.

P27-54
Metabolites of a dietary supplement: the case of *Vaccinium macrocarpon*

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Food supplements contain numerous natural plant constituents and herbal extracts with proposed beneficial effects for individual's health. They are often considered harmless as based on natural plant extracts and compounds. However, many problems may be associated with their use, including potential adverse effects and food supplement–drug interactions.

The aim of this preliminary study was to investigate the biotransformation of the active component of a nutritional supplement based on cranberries (*Vaccinium macrocarpon* Ait., Ericaceae), recommended to prevent urinary tract infections. A commercial cranberry product HI-PAC 4.0 (spray-dried fruit juice standardised to 4.75% proanthocyanidins and containing 6.59% phenolic and other organic acids, 2.18% quercetin and 0.61% anthocyanins) was incubated with (i) liver microsomes from rats treated with phenobarbital or β-naphtoflavone and from control rats and (ii) with rat hepatocytes in suspension. The samples were analyzed using μLC-DAD-MS for metabolites.

No metabolites assignable to proanthocyanidins were found. An increase in response was noted for the ions m/z 153 (negative ionisation), corresponding to dihydrobenzoic acids. Hippuric acid was found among metabolites after treatment using rat hepatoctes. Hydroxylated quercetin was not found at all and only low signal of ion at m/z 333 was observed in samples after treatment with microsomes. Beside ion at m/z 477 was found in metabolized HI-PAC sample at negative ionisation which was not detected in appropriate control sample. This ion can be tentatively assigned to methylquercetin-glucoside.

Detailed investigation of metabolic processes in rat hepatocytes is in progress.

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P27-55
Production of carbohydrate-rich microalgal biomass coupled to photosynthetic CO_2 abatement

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CO_2 is photosynthetically fixed and converted into organic matter by microalgae and cyanobacteria. Part of the resulting biomass can be used as raw material for transformation into valuable biofuels, such as ethanol or biodiesel. The selection of microalgal strains for the purpose of CO_2 abatement coupled to renewable biofuel production should take into account parameters as CO_2 fixation rate, biomass productivity and carbohydrate and fatty acid content, as relevant selection criterions. The continuous culture approach has been undertaken to assess the potential of a variety of microalgal strains.

A screening has been performed using 1.8 l photochemostats operated at a dilution rate of 0.3 per day, pH 7.5 and 20°C. The highest value of CO_2 fixation capacity (close to 2 g CO_2 per l/day) was found for *Chlorella vulgaris* cultures, which were able to generate 86 mg carbohydrates per l/day, from which 29% corresponded to starch (25 mg starch per l/day). Under conditions of moderate nitrogen limitation (5 mM nitrate in fresh medium) the productivity values for carbohydrates and starch were increased to 146 and 59 mg per l/day, respectively, with starch representing 41% of total carbohydrates. A further reduction in nitrate availability did not result in enhanced starch productivity, although the cellular level of the polysaccharide increased up to 44% of total carbohydrates.

Thus, *Chlorella vulgaris* represents a suitable candidate for their use in CO_2-abatement microalgal systems coupled to the production of a biomass with substantial heat of combustion (20 kJ/g) and high content in starch, raw material for fermentative ethanol production.

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P27-56
Id1B, an alternatively spliced isoform of the Inhibitor of differentiation-1, impairs cancer cell malignancy through inhibition of proliferation and angiognesis

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Id-1, a member of the HLH family, regulates the activity of transcription factors to suppress cellular differentiation and promote cell growth. Overexpression of Id-1 in tumor cells correlates with increased malignancy and resistance to therapy.

Splicing is a molecular phenomenon that allows the generation of multiple mRNAs encoding functionally different protein isoforms. Concerning Id-1, Id-1B is an isoform generated by alternative splicing that differs from the classical Id-1 in the 13-C-terminal aminoacids. At present Id-1B is largely unknown. Since alternative splicing may generate mRNAs encoding functionally different proteins, we therefore studied the role of Id-1B in cancer as compared to classical Id-1, a protumorgenic factor. We overexpressed Id-1B in A549 and PC3 cells to study its role in lung and prostate cancer, respectively. Cell clones stably transfected to overexpress Id-1B showed a significantly reduced proliferation activity and clonogenic potential than control cells. Moreover overexpression of Id-1B caused the accumulation of cells in the G1 phase, as well as the increase of p57 and p27, whereas levels of p-Erk and cyclin A were reduced. Moreover, Id-1B overexpressing cells treated with 4Gy irradiation dose were significantly less resistant to cell death. *In vivo* assays demonstrated that tumors exhibiting high level of Id-1B show significantly more survival, less metabolic activity and less angiogenesis compared to those with low expression. In addition, we quantified by real time RT-PCR expression levels of Id-1 total and Id-1B in 17 non-small cell lung cancer specimens and their matched non-malignant counterparts. Expression levels of Id-1 did not change. On the contrary, a significant downregulation in the expression of Id-1B was found in tumors with respect to non-tumor samples. Id-1B may then contribute to impair cellular malignancy.

P27-57
An investigation on the antioxidant activities and total phenolics content of *Calendula officinalis*

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Plants are the best source of active secondary metabolites which are beneficial to mankind. Many plant origin drugs have been reported with biological properties. *Calendula officinalis* is an important medicinal plant with various bioactivities. In this study, ethanol extract of *Calendula officinalis* flowers obtained from herbal shops was evaluated for their antioxidant capacity using DPPH and ABTS methods. Fifty percent effective concentration (EC_{50}) values for the extract was found as 2.33 mg/ml according to radical scavenging activity (RSA) in percentage resulting from DPPH scavenging study. The antioxidant capacity value was also examined with ABTSand determined according to Trolox equivalent antioxidant capacity (TEAC) value wich was calculated as 72 μM/mg extract. At the same time, total phenol (TP) quantification of the extract was obtained by assessing gallic acid equivalent. TP content of *C. officinalis* was obtained as 7.5 μg GAE/mg. This is the first study as the evaluation of the antioxidant capacity of *C. officinalis*.

Keywords: antioxidant activity, C. *officinalis*

P27-58
Purification and characterization of the recombinant human prostaglandin H synthase-2 expressed in *Pichia pastoris*

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Prostaglandin H synthase-1 and -2 (PGHS-1 and PGHS-2, EC 1.14.99.1) are membrane associated glycoproteins that catalyze the first two steps in prostaglandin synthesis. As the enzymes play an important regulatory role in several physiological and pathophysiological processes, recombinant PGHS isoforms are widely used in biomedical research. In the present study, we expressed human PGHS-2 (hPGHS-2) with and without a six histidine sequence tag (His_6 tag) near the amino- or carboxy-terminus of the protein in the *Pichia pastoris* (*P. pastoris*) expression system using native or yeast signal sequences. The recombinant His_6 tagged hPGHS-2 was purified using Ni-affinity and anion exchange chromatography, whereas the purification of the C-terminally His_6 tagged hPGHS-2 was more efficient. K_m, k_{cat} and IC_{50} values were determined to characterize the protein. The data obtained indicate that both the N- and C-terminally His_6 tagged hPGHS-2 are functional and the catalytic properties of the recombinant protein and the enzyme produced in other expression systems are comparable. As the yeast culture is easy to handle, the *P. pastoris* system could serve as an alternative to the most commonly used baculovirus-insect cell expression system for the production of the recombinant PGHS-2.

P27-59
Differential roles of coatomer isoforms?

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Coatomer is a molecular coat whose polymerization on organelle membranes induces the formation of COPI vesicles. It is composed of seven subunits, two of them present as two isoforms, leading to four different coatomer isotypes in mammalian cells. Coatomer shares mechanistical similarity and structural analogy with the well-characterized clathrin system. Different adaptors allow recruitment of the clathrin coat to different organelle membranes and selection of different sets of cargos for inclusion into coated vesicles. COPI vesicles have been reported to be involved in different trafficking steps within the Golgi apparatus as well as the ER-Golgi interface. In accordance with this hypothesis, different subpopulation of COPI vesicles with distinct cargo protein compositions have been isolated. Interestingly, electron microscopy analysis revealed a differential localization of coatomer isotypes along the Golgi stacks. It is thus tempting to speculate that, like for adaptins, different coatomer isotypes provide ways to modulate the cargo repertoire of the COPI system, leading to distinct pools of coated vesicles involved in different pathways.

To assess this hypothesis, the four coatomer isoforms are expressed recombinantly and subjected to Golgi membrane for *in vitro* budding assays. COPI vesicles are then purified on sucrose gradient and analyzed for their lipid and protein compo-

sitions. Taking benefit of both Western-Blot and recently developed label-free quantitative proteomic approaches, we identified several cargos that differentially incorporate into different isotypic COPI vesicles.

P27-60
APC15 drives the turnover of MCC-Cdc20 to make the spindle assembly checkpoint responsive to kinetochore attachment

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Faithful chromosome segregation during mitosis depends on the Spindle Assembly Checkpoint (SAC) that monitors kinetochore attachment to the mitotic spindle. How the SAC remains highly responsive to the state of the kinetochores is important to understand genomic stability.

A single unattached kinetochore is able to delay cells in mitosis but once it attaches the cell rapidly enters anaphase. The SAC sequesters Cdc20 into the MCC initially composed of the Mad2, BubR1 and Bub3 checkpoint proteins, which accumulate at unattached kinetochores. This blocks the ubiquitylation of Cyclin B1 and securin until all kinetochores are attached and thereby prevents sister chromatid separation and exit from mitosis. How the MCC binds to the Anaphase Promoting Complex/Cyclosome (APC/C) and how it is released from the APC/C after the SAC has been satisfied is a matter of debate.

To identify interactors of the APC/C that promote the release of the MCC we compared APC/C purified from different stages of mitosis by quantitative mass spectrometry. We identified a novel subunit of the human APC/C, APC15, which is crucial for SAC function but is not required for APC/C activity *per se*. Depleting APC15 prevents Cyclin B1 ubiquitylation and degradation because the APC/C activator Cdc20 remains locked onto the APC/C in complex with inhibitory mitotic checkpoint proteins, which prevents APC/C activation when the SAC is satisfied. We conclude that the constant turnover of MCC's at the APC/C by APC15 allows the SAC to respond to the attachment state of kinetochores.

P27-61
OPDA isomerization and insect development: hint of bifunctionality in (Glutathione S-transferase) GST16 enzyme

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12-oxophytodienoic acid (OPDA), a well-known phytohormone of the jasmonate family has a reactive α, β-unsaturated carbonyl structure, which easily adds cellular nucleophiles (Michael addition), making OPDA potentially toxic for herbivores. OPDA is isomerized into the inactive iso form by a specific glutathione S-transferases (HarmGST16) present in *Helicoverpa armigera.* Quantitative tissue expression analysis showed that HarmGST16 transcripts are present in most larval tissues, including the midgut, fatbody, and malpighian tubules and enzyme activity studies confirmed the OPDA isomerization activity in the same tissues. Interestingly, feeding of different diets to *Helicoverpa armigera* influences GST16 expression levels in various tissues, and larvae fed on wild type tobacco leaves have reduction in GST16 mRNA level. Temporal expression during development showed high expression of HarmGST16 in the second instar followed by descending expression during the third, fourth and fifth instar. Plant mediated RNAi silencing of HarmGST16 shows growth retardation in Helicoverpa armigera . The injection of *cis*-OPDA in the hemolymph of *Helicoverpa armigera* larvae resulted in prepupation of larvae. At the same time, considerable number of pupa with earlier pupation developed into malformed adults. This result, in conjugation with finding that GST16 can influence growth of insects, it suggests that GST16 may has an important role in development. Isomerization of OPDA is one of the important tasks related to this enzyme.

P27-62
The effect of smoking on the level of urine citrate in pregnant women

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Urolithiasis is the most frequent disease of urinary system. Citrate is an inhibitor of the crystallization of stone-forming calcium salts. The aim of this study was to evaluate the level of urine urine citrate in the smokers and non smokers pregnant women. Eighty-six pregnant women (10 smoker, 10 nonsmoker) were participated in the study and measured the citrate level of them. While the level of urine citrate in smokers has been found 382.82 ± 273.44 mg/l, in non-smokers has been found 510.01 ± 264.09 mg/l. In pregnant, smoking causes a decrease in the value of urine citrate, it increases susceptibility to the formation of kidney stones.

P27-63
Hematologic, biochemical and antioxidant properties of hydromethanolic extract of *Senna alata* and *Senna podocarpa* leaves on albino rats

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Senna alata and *Senna podocarpa* are medicinal plants widely used in complementary and alternative medicine in the treatment of constipation, hemorrhoids, gonorrhea as well as other diseases, in southwestern Nigeria. The effect of hydromethanolic leaf extract of *S. alata* and *S. podocarpa* on hematologic, biochemical, and antioxidant indices was assessed in normal albino rats. Dried leaves of *Senna alata* and *Senna podocarpa* were extracted in cold aqueous methanol and dried under vacuum. Thirty-five (35) male *Rattus Novergicus* distributed into seven groups comprising of five rats each, administered 1 ml daily gavage distilled water to the control group while the extract at a dose of 200, 600 and 1000 mg/kg body weight for 63 days. Catalase activity, concentration of thiobarbituric acid reactive substance (TBARS) as well as some blood parameters was monitored using standard protocols. Both *Senna alata* and *Senna podocarpa* extract significantly ($p < 0.05$) elevated the hematocrit level at all doses tested, however only the monocytes were significantly ($p < 0.05$) elevated at all doses of *S. alata.* The albumin and total bilirubin levels were

not significantly ($p < 0.05$) affected by both extracts, but in the group that received the 600 mg kg per bwt of the *S. alata* extract, the total protein level was significantly ($p < 0.05$) elevated. A significant ($p < 0.05$) reduction in plasma alanine aminotransferase (ALT) was observed in the rats that received 1000 mg kg per bwt of *S. alata*, whereas alkaline phosphatase (ALP) was significantly ($p < 0.05$) elevated in the rats administered 600 mg kg per bwt of *S. podocarpa*. Plasma lipid peroxidation was significantly decreased at all doses of extract tested except in the rats that received 600 mg kg per bwt of *S. alata,* while liver lipid peroxidation reduced significantly in the rats at all doses of *S. alata*. Plasma catalase activity on the other hand was not significantly affected whereas liver catalase activity was significantly decreased only in rats that received 200 mg kg/bwt of *S. alata*. The results from this study may suggest a possible role of these extracts in the management and improvement of anemic conditions, while the TBARS reducing capability of the extract may reveal some intrinsic antioxidant properties that may combat oxidants involved in lipid peroxidation.

Keywords: Antioxidant, *Senna alata*, *Senna podocarpa,* Catalase, TBARS level, Hematologic

P27-64
GEFs determine the intracellular organelle localization of Rab GTPases

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Membrane fusion at eukaryotic organelles is initiated by Rab GTPases and tethering factors. Rabs in their GDP-form are kept soluble in the cytoplasm by the GDI chaperone. Guanine nucleotide exchange factors (GEFs) are found at organelles and are critical for Rab function. Here, we surveyed the overall role of GEFs for Rab localization. We show that GEFs, but none of the proposed GDI displacement factors (GDFs), are essential for the correct membrane localization of Rabs. In the absence of the GEF, most Rabs redistribute to the ER, which presumably serves as a reservoir compartment, even in the presence of excess GDI. Our data agree with the emerging model that GEFs are sufficient to displace GDI and confer activation and membrane localization of Rab GTPases.

P27-65
Interaction of anticancer drug, etoposide, with chromatin proteins, in solution

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Etoposide is a highly successful anticancer agent that has been used to treat a variety of blood-born and solid human malignancies for nearly 30 years. The drug is a derivative of podophyllotoxin, a naturally occurring antimitotic agent from may apples. Although its molecular target is DNA topoisomerase II, the interaction between etoposide and nuclear protein such as histones is not understood. In this study, for the first time we have investigated the effect of this drug on histone proteins, H1 and core histone, employing fluorescence, UV/Vis, CD spectroscopy techniques. The results showed that etoposide at low concentration increased the absorbances of H1 and core histone at 210 nm whereas at higher concentration induced hypochromicity. Fluorescence emission intensity of histone H1 and core histone, was increased when 5 μM of drug is used but at higher concentration (> 5 μM) etoposide decreased emission intensity of these proteins without any red shift in the emission maximum. Binding of etoposide changed secondary structure of the histone proteins as circular dichroism confirmed it. Addition of etoposide to H1 solution caused a significant reduction in the ellipticiy without any change on the negative extreme at 198 nm. In the case of core histone, etoposide decreased ellipticity and showed no change on both negative extreme at 206 and 222 nm. The results suggest that apart from topoisomerase II as a main target for etoposide, histone proteins can also be considered as a target for etoposide in the nucleous.

Keywords: etoposdie, Chromatin, histone proteins.

P27r-66
Functional role played by ARFGAP1 at the ergic compartment

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Assembly of COPI- and clathrin-coated vesicles depends on the small GTPase ARF1 whose activity is regulated by both guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Although ARFGAP1 was initially described to be responsible of ARF1 inactivation recent data indicate that this function is mostly performed by ARFGAP2/3. Thus the functional role of ARFGAP1 is uncertain at present. In mammalian cells the three ARFGAP isoforms are primarily localized to the Golgi complex. In addition, ARFGAP1 but not ARFGAP2/3 is localized at elements of the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC). We have investigated the functional significance of ARFGAP1 localization at ERGIC. Association to ERGIC was independent of COPI since it was not affected by pretreatment with Brefeldin A which causes dissociation of this coat. Conversely, COPI binds ERGIC in cells lacking ARFGAP1 by siRNA treatment. However, ARFGAP1 overexpression gave rise to COPI dissociation. Furthermore, under these conditions numerous vesicle buds were seen protruding from ERGIC membranes. Together, these data suggest the existence of a regulatable balance between both ARF1 and ARFGAP1 activities aimed to control vesicle production at ERGIC. We also report that under steady-state conditions ARFGAP1 is phosphorylated at serine 360 by PKA activity. At present we are examining the effects of this modification on the dynamic behavior of ERGIC elements.

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P27-67
Role of a novel protein in alveolar bone remodeling during orthodontic tooth movement

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Phospholipase C-related but catalytically inactive protein (comprising PRIP-1 and -2) was isolated as a novel inositol 1,4,5-trisphosphate binding protein with a domain organization similar to phospholipase C-delta but lacking the enzyme activity. We have recently reported that PRIP-1 and -2 double knockout (DKO) mice showed up-regulation of gonadotropin secretion, albeit with lower levels of serum sex steroid hormones. Therefore we analyzed the bone properties of PRIP-DKO mice and found that the bone mineral density and trabecular bone volume were up-regulated in PRIP-DKO mice, due to enhance bone formation and decreased bone resorption. Orthodontic tooth movement is

achieved by the process of alveolar bone resorption on the pressure side and new bone formation on the tension side. In this study, we investigated the possible involvement of PRIP in alveolar bone remodeling during orthodontic tooth movement. To achieve the upper molar movement, a closed coil spring was inserted between the upper incisors and the upper first molar in both genotype of mice, wild-type and PRIP-DKO. In PRIP-DKO mice, tooth movement appeared to be reduced compare to the wild type mice assessed by morphological analysis using micro CT. Histological analysis using alkaline phosphatase and tartrate-resistant acid phosphatase staining showed the difference in distributions of osteoblasts on the tension side and osteoclasts on the pressure side of alveolar bones, respectively. These results indicate that PRIP is implicated in regulation of bone remodeling during mechanical tooth movement.

P27-68
Novel cytostatic agents based on bile acid – hydrazone conjugates

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Hydrazones are class of compound with wide range of biological activity. Suitable substituted hydrazones, such as heteroaryl hydrazones and aroyl hydrazones derived from 2-hydroxyaryl- or 2-(N)-heteroaryl carbonyl compounds have ability to bind biological important metal ions (namely Fe, Zn and Cu) by NNO or NNN binding system. These metal ions play a crucial role in many biochemical processes. Hydrazones functioning within the meaning of metal chelators were already successfully used for binding of iron in biological systems. Recent studies indicate that some hydrazones showed significant anticancer activity.

Combination of hydrazone unit (part with biological activity) with bile acids provides conjugates with improved properties for their utilization as cytostatic agents. Bile acid unit will improve cell membrane permeation, solubility and thus increase bioavailability of therapeutic unit.

Anticancer activity of prepared conjugates were tested on human promyelocytic leukemia cells (HL60) and mammary carcinoma cells (4T1). The most active compounds have IC50 values lower than 3 μM.

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P27-69
Antiplasmodial activity of Nerolidol in Balb/c mice infected with *P. berghei*

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Malaria represents a major public health problem, especially for the underdeveloped and developing countries. The increasing resistance of malaria parasites to almost all available drugs calls for the identification of new compounds and the detection of novel targets. Previously, we have demonstrated *in vitro* that some terpenes inhibit the erythrocytic stage of *P. falciparum* by decreasing some products of the isoprenoid pathway. To confirm that effect *in vivo*, we investigated the potential antimalarial activity of Nerolidol, a sesquiterpene, in malaria-bearing mice. Balb/c mice were infected with *P. berghei* and treated with Nerolidol at a dose of 400 mg/kg/day orally or by inhalation for 7 days, followed by quantification of parasitemia and plasma levels of Nerolidol by optical microscopy and gas chromatography-mass spectrometry, respectively. Interestingly, a marked decrease in parasitemia was demonstrated with Nerolidol treatment. Oral administration of Nerolidol inhibited 54% parasitemia, while the inhalation via showed an inhibition of 48%. According to that, both via of administration were able to increase significantly the survival of infected animals in 50%. Moreover, Nerolidol showed no toxicity in mice as evaluated by LD_{50} (> 5000 mg/kg) and serum levels of ALT, AST and creatinine. In addition, we are developing a sensitivity method to determine the Nerolidol concentration in mice plasma at ng/ml using a gas chromatographic–mass spectrometric analysis. Our study represents the first evidence of Nerolidol as antiplasmodial activity *in vivo*, probably by inhibition of isoprenoid pathway in the parasites, besides indicating the inhalation as a promising via of administration for antimalarial drugs.

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P27-70
Synthesis and anticancer activity of novel phthalazinyl hydrazones

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Heteroaryl hydrazones are class of compounds with significant biological activity and many of these compounds display anticancer activity. Therefore we designed and synthesized set of phthalazinyl hydrazones for testing their activity against cancer cells. Anticancer activity evaluation on the human promyelocytic leukemia cells (HL60) and mouse mammary carcinoma cells (4T1) showed that some phthalazinyl hydrazones have significant inhibitory effect against both cancer cell lines. Complexation studies toward biologically important metal ions at biologically relevant conditions show general ability to bind Cu^{2+}, Co^{2+}, Ni^{2+} and Fe^{3+} (with some exceptions) and rarely Zn^{2+} and Fe^{2+}. There is not any clear correlation of binding ability with anticancer activity; however all derivatives able to bind Zn display very high activity (IC_{50} < 1 μM) and opposite way all derivatives without binding ability towards Co do not display any significant activity (IC_{50} > 10 μM). Hydrazones are known to display tautomerism; QD/MD calculations in aqueous media show preference of hydralazine form. Calculations also show that metallo-complexes of derivatives are relatively planar and thus potentially allow intercalation into DNA in contrast to derivatives themselves. This is in good agreement with experimental observation that metallo-complexes of many derivatives display ability to interact with DNA but derivatives themselves do not.

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P27-71
A cell specific strategy using high content bio-imaging for discovery of novel nuclear export machinery antagonists from natural product of microbial origin

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Natural products have largely been, and still are, considered an exceedingly valuable platform for the discovery of new drugs against diverse pathologies. As yet we have only explored a fraction of the diversity of bioactive compounds, so opportunities for discovering new natural product leads for new drugs are huge. A main objective of Fundación MEDINA is the discovery of novel drugs of microbial origin particularly for tumoral processes. Fundación MEDINA's natural products collection with 100 000 microbial strains and 130 000 extracts covers an uncommonly broad chemical space. Intracellular localization is essential for the regulated activity of many signaling molecules associated with cancer-relevant pathway. In this project a cell-based system for the identification of nuclear export inhibitors using GFP labeled Rev, which contains a strong heterologous NES (leucine-rich Nuclear Export Signal) protein was applied to screen a collection of extract from microbial origin. The fluorescent signal of untreated U2nesRELOC cells localizes exclusively to the cytoplasm. Upon treatment with the nuclear export inhibitor reporter protein accumulates rapidly in the cell nucleus. A selected part of MEDINA collection from fungi, actinomycetes and non cultivable bacteria were tested. A two stapes analysis with high content bio-imaging allowed us to select extracts with biological activities which are not associated with previously known active metabolites. The fractionation and structural elucidation of active compounds are in progress. This project demonstrated that we can apply a natural product extract collection from microbial origin to a cell-based nuclear export assay in a high throughput screening way to detect novel nuclear export inhibitors.

P27-72
Assessment of metallothioneins in tissues of marine invertebrates as biomarkers for environmental heavy metals pollution: seasonal and spatial variability

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During the recent years several reports have been published describing seasonal and spatial variability of metallothioneins (MT) concentrations in marine organisms as biomarkers for heavy metal pollution (1). The most popular marine organisms utilized in monitoring programs are the marine mussels *Mytilus* spp. (2, 3, 4). Unfortunately this specie is not represented or cultivated along the cost of Tuscany. Therefore, we decided to select a new sentinel organism. MT levels has been determined spectrophotometrically (5) in the soluble fraction of the gonadic tissue of *Paracentrotus lividus* and of the whole organisms of *Monodonta turbinata* and *Patella* spp. Specimens were collected between 2011 and 2012 from several coastal locations along the North-Tyrrhenian coasts (Tuscany, Italy). The seasonal influence on the MT (higher values in winter-spring season, than in summer-autumn season) is more pronounced than the local-specific influence. Furthermore, within each season a significant site-specific dependence on the MT can be detected. The seasonal variability indicate that reproductive cycle and abundance of food are the major determinants for the metallothioneins expression in the three organisms selected. Nevertheless, spatial variability indicate an increase of metallothioneins expression from North to South according to the semestral report of heavy metal pollution of the local control agency. In conclusion, *Monodonta turbinata* has been found to represent an optimal heavy metal pollution biomarker, answering very sensibly to the environmental fluctuations, so then, chronic and acute toxicology tests are in progress in order to quantify the gastropod capacity to detoxify the environment from heavy-metals through MT expression.

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P27-73
Human coronavirus NL63 nucleocapsid protein stimulates an immune response in whole blood culture

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Human coronavirus-NL63 (HCoV-NL63) is closely related to human severe acute respiratory syndrome coronavirus (SARS-CoV), but unlike SARS-CoV, continuously circulates in the human population. HCoV-NL63 has been detected in 1–9% of respiratory samples collected worldwide and is associated with both upper and lower respiratory tract infections. Coronavirus nucleocapsid (N) protein is a multifunction protein and plays an important role in viral assembly, pathogenesis and antibody production. Recent studies have focused on the effect of coronavirus infections on the host immune response, in particular the production of cytokines. In this study, the effects of HCoV-NL63 N on the cytokines, that regulate the inflammatory and anti-inflammatory immune responses in lymphocytes, were determined. Full-length HCoV-NL63 N, as well as deletion-mutants, were cloned, expressed in a bacterial expression system and column-purified. Cellular responses induced by these recombinant N proteins were then evaluated in whole blood culture. A double antibody sandwich ELISA assay was used to measure the responses of inter-

feron-gamma (IFN-γ), interleukin-10 (IL-10) and interleukin-6 (IL-6) against N. While lymphocyte activation resulted in high expression levels of IL-6, IFN-γ and IL-10 cytokines were secreted at much lower levels. This study showed that the HCoV-NL63 N protein elicited a broad based cellular immune response.

P27-74
Characterization of Co(II) biosorption by *Schizosaccharomyces pombe*

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Improper treatment and disposal of metal-contaminated wastewaters raise concerns for soil and water contamination. Among those metals cobalt element is widely used in modern industry due to many physical and chemical characteristics peculiar to it. Cobalt mining, alloy production, electroplating, generation of gas turbines, and petrochemical industries' wastewaters contain large amounts of Co(II). It is imperative to implement effective and feasible removal methods at the source of heavy metal pollution. The utilization of microorganisms as biosorbents for the removal and recovery of heavy metals from industrial wastewaters has become a major alternative to conventional methods. Microorganisms, active or inactive, can adsorb dissolved metals by the courtesy of their special membrane characteristics. In this study, the potential of the dried yeast, wild-type *Schizosaccharomyces pombe*, to remove toxic Co(II) ion from the solution phase was investigated in batch mode by changing one experimental condition at a time. Those conditions include solution pH, temperature, initial metal ion concentration and biosorbent dose. Optimum pH for Co(II) biosorption was determined as 5.0 and all experiments were conducted at that pH. Equilibrium between the adsorbed phase and solution phase was attained within 2 hours. The equilibrium uptake decreased with increasing temperature denoting an exothermic behavior. Several isotherm models including Langmuir and Freundlich isotherms were used to evaluate the equilibrium data. Reaction rate and thermodynamic properties of Co(II) biosorption at 20, 25, 30, 35 and 50°C were also determined. Funding by M.U. Nihad Sayar Egitim Vakfi and M.U. Research Fund Project FEN-C-YLP-010710-0231 are gratefully acknowledged.

P27-75
Synthesis and antidiabetic activity of some novel substituted-chromonyl-2,4-thiazolidinedione derivatives

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Diabetes mellitus (DM) is increasing in prevalence worldwide, a condition characterized by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from the pancreatic beta cells (1). Thiazolidinediones (TZDs) or glitazones, ligands of the peroxisome-proliferator–activated receptor-γ, which are a class of antihyperglycemic agents that decrease insulin resistance and improve insulin action, thereby improving glycemic control and potentially preserving beta-cell function (2).

There are two widely used commercial products derived from TZD, rosiglitazone (Avandia), and pioglitazone (Actos). The known risk profile for TZD includes weight gain, fluid retention, and, in some patients, a worsening of congestive heart failure (3). On September 23, 2010, the FDA announced their decision to require a Risk Evaluation and Mitigation Strategy for rosiglitazone, which will limit its availability. In US, rosiglitazone was removed from the retail pharmacy stores by November 18th in 2011. In this case, pioglitazone is the only TZD-based drug available in the US and European markets (4).

With prevalence of type 2 diabetes and limited number of insulin sensitizers, there is a huge demand for a new drug in safety. For these reasons, significant efforts are ongoing to develop novel TZDs, which retain their insulin-sensitizing activity and are devoid of activities that cause adverse effects.

In this study, in our screening program to search for antidiabetic compounds, the 2,4-TZD, imidazolidine-2,4-dione and 2-thioxo-imidazolidine-4-one containing substituted chromone ring were synthesized and their insulin releasing activities in INS-1 cells were evaluated.

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P27-76
The role of MDR1 C3435T single nucleotide polymorphism in male infertility

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Introduction: Infertility is a common problem, affecting one in six couples. In 30% of infertile couples, the male factor is a major cause due to defective sperm quality. However, the factors responsible for defective sperm quality remain largely unknown.The multi-drug resistance1 (MDR1) gene encoding a P-glycoprotein (P-gp), which has a role in active transport of various substrates, including xenobiotics, and thus has a protective function in various tissues and organs. In the present case-control study, we investigated the effect of MDR1 gene C3435T SNP and on male infertility in Turkish population.

Materials and methods: The study was performed on 143 patients with infertil and 106 healthy fertile control subjects. The genotyping of C3435T SNP was done by PCR-RFLP. In statistical analysis allele and genotype frequencies were tested with chi-square test.

Results: The frequencies of C and T alleles were 51.7% and 48.3% in patient group, whereas 50.5% and 49.5% in control group respectively. The allele frequencies in patient and control groups were found to be similar ($p > 0.005$). Genotype frequency distribution of C3435T polymorphism in patient group was CC: 24.5%,CT: 51.9%, TT: 23.6% and in control group was CC: 25.2%, CT: 53.1%, TT: 21.7%. Genotype frequencies didn't show significant difference between patient group and control subjects ($p > 0.005$). Conclusion: To our knowledge this study is the second study about MDR1 gene polymorphism and male infertility in literature and first study in Turkish population. Our up to date findings show that C3435T SNP does not play a role in the genetic susceptibility to male infertility, controversial to Polish population (Drozdzik et al.2009).

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P27-77
The yeast mitogen-activated protein kinase Slt2 is involved in the cellular response to genotoxic stress

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The maintenance of genomic integrity is essential for cell viability. Complex signalling pathways (DNA integrity checkpoints) mediate the response to genotoxic stresses. Identifying new functions involved in the cellular response to DNA-damage is crucial. The *Saccharomyces cerevisiae SLT2* gene encodes a member of the mitogen-activated protein kinase (MAPK) cascade whose main function is the maintenance of the cell wall integrity. However, different observations suggest that *SLT2* may also have a role related to DNA metabolism. This work consisted in a comprehensive study to connect the Slt2 protein to genome integrity maintenance in response to genotoxic stresses. The *slt2* mutant strain was hypersensitive to a variety of genotoxic treatments, including incubation with hydroxyurea (HU), methylmetanosulfonate (MMS), phleomycin or UV irradiation. Furthermore, Slt2 was activated by all these treatments, which suggests that Slt2 plays a central role in the cellular response to genotoxic stresses. Activation of Slt2 was not dependent on the DNA integrity checkpoint. For MMS and UV, Slt2 activation required progression through the cell cycle. In contrast, HU also activated Slt2 in nocodazol-arrested cells, which suggests that Slt2 may respond to dNTP pools alterations. However, neither the protein level of the distinct ribonucleotide reductase subunits nor the dNTP pools were affected in a *slt2* mutant strain. An analysis of the checkpoint function revealed that Slt2 was not required for either cell cycle arrest or the activation of the Rad53 checkpoint kinase in response to DNA damage. However, *slt2* mutant cells showed an elongated bud and partially impaired Swe1 degradation after replicative stress, indicating that Slt2 could contribute, in parallel with Rad53, to bud morphogenesis control after genotoxic stresses.

P27-78
Remazol brilliant blue royal biodegradation catalyzed by methylsyringate-laccase mediator system

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Remazol Brilliant Blue R (RBBR) is an anthraquinone dye used as starting material in the production of polymeric dyes. Two percent of produced dyes are discharged directly into the aqueous effluents. RBBR is toxic, mutagenic and carcinogenic; thus it is necessary to degrade it from wastewater. It is studied and optimized the biodegradation of RBBR by the oxidative enzyme Laccase from *Trametes villosa* (TvL, Novozymes) and the natural mediator Methyl Syringate (MSG). Chemical effects (pH and amount of enzyme/substrate/mediator) were carried out in the spectrophotometer with microplate reader of 96 wells from Molecular devices at 600 nm and 25°C. The identification of the reaction components (reagents and product) was done on a HPLC Agilent 1200 Rapid Resolution with analytical reverse phase and photodiode array detector run on Agilent ChemStation software. The HPLC column was a Zorbax Eclipse C18 600 bar with 4.6 × 50 mm and 1.8 mm of particle. A blue decolouration percentage of 81% was attained in < 30 min for the degradation of 24 mM RBBR by 359 nM TvL (900 EU/l) and 2.5 mM MSG in 10 mM acetate buffer pH 6.0. A slightly brownish colour was formed and the products were investigated using the HPLC, which resulted in an increase in the absorbance in the range 400–500 nm, similar to previous results and concluding that the intermediate product should be 2,2′-disulfonyl azobenzene. Thus, it is presented a green, cheap and fast alternative for the degradation of RBBR by Laccase, an enzyme widely available from fungi. This work has been partially supported by grants from several Spanish organizations. Projects BIO2009-12956 (MICINN, Madrid) and 08856/PI/08 (Fundación Seneca, CARM, Murcia). Predoctoral fellowship MP 09378/FPI/08 (Fundación Seneca, CARM, Murcia).

P27-79
Effect of the AMPK activator metformin in physiological processes of boar spermatozoa necessary to accomplish oocyte fertilization

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The sensor molecule that detects cell energy state and regulates metabolism is AMP-activated kinase, AMPK. We recently showed that AMPK is highly expressed in boar spermatozoa and regulates motility, necessary process to achieve oocyte fertilization. A known AMPK activator is Metformin (Metf). To date, all Metf studies were performed in somatic cells, therefore our aim is to study Metf effects in physiological parameters indicative of the spermatozoa functional state. Cell viability, plasma membrane scrambling, mitochondrial membrane potential (MMP) and the functional status of the acrosome were analyzed by flow cytometry after incubation of spermatozoa in presence/absence of Metf for different times (2, 24 hour) and concentrations (1–20 mM). Metf treatment significantly decreases the percentage of spermatozoa with high MMP in a concentration and time-dependent manner, being totally reduced at 5 mM (24 hour). In parallel, Metf significantly increases the percentage of acrosome-damaged spermatozoa in a concentration and time-dependent manner (until 50% at 24 hour, 5 mM). Moreover, 5 mM Metf causes slight plasma membrane scrambling in a time-dependent manner. Spermatozoa viability or acrosome-reaction induced in capacitated spermatozoa by the calcium ionophore A23187 are unaffected by Metf. Our data show that Metf negatively affects high MMP and acrosome status, physiological parameters indicative of an optimal spermatozoa functional state. Supported by National MICINN (AGL2010-15188) and Regional Grants GR10156-JUEX. David Martín-Hidalgo is recipient of a PhD fellowship from JUEX, Spain.

P27-80
Neurotrophin 3 – adaptation marker to chronic and acute brain hypoxia

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Background: In acute ischemia (ischemic stroke-i.s), early excitotoxicity can lead to fast necrotic cell death, which produces the core of infarction. While brain cells are challenged by this deleterious mechanisms, they activate innate protective programs of the brain, including synthesis of inflammatory cytokines and neuronal growth factors, members of the neurotrophins family, suggesting that neuronal death is associated with an inflammatory reaction and a protective response. Chronic obstructive pulmonary disease (copd) is a major cause of chronic hypoxia and cerebral ischemia, involving a complex signaling cascade with at least one partially unraveled spatiotemporal pattern. In chronic brain hypoxia, the intensity of the inflammatory reaction and the efficiency of the protective response are poorly characterized. The aim of this work is the completion of the comparative study between patients with copd (chronic brain hypoxia) and patients with ischemic stroke (acute brain hypoxia), regarding the neurotrophin-3 serum level, in order to establish a possible model of protective brain response to hypoxia.
Patients and methods: Sixty-eight patients were investigated after computered tomography-confirmed i.s. We used Elisa to determine the serum level of neurotrophin-3 (as marker of the brain adaptation response). The results were compared with those obtained in 56 patients with confirmed copd. Results: a significant increase of neurotrophin-3 serum levels was obtained in copd compared with normal and ischemic stroke lots. Conclusion: increased neurotrophin-3 serum level could represent an important marker of neuroprotection, this neurotrophic factor being a potential therapeutic agent in patients with acute or chronic brain hypoxia.

P27-81
Searching for the coding sequence of the CCAP receptors in the genome of ageing Tenebrio molitor beetles

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The crustacean cardioactive peptide (CCAP; PFCNAFTGCa) has been identified in neurohormonal system of many insect species including the beetle *Tenebrio molitor*. It exerts, inter alia, cardiotropic activity, and can modulate the action of insect heart. The peptide has been shown to induce an increase in the frequency and amplitude of contraction of the *T. molitor* myocardium. It can act on cells via G-coupled receptor, which has homology to vertebrates NPS receptor. In the most related species *Tribolium castaneum*, two CCAP receptors were detected. It was also shown that CCAP activates both receptors in *T. castaneum* in a heterologous expression system. RNA interference (RNAi) of CCAPR-1 and CCAPR-2 revealed that only CCAPR-2 mediates the cardioacceleratory function and is essential for eclosion behavior in *T. castaneum*.

In our previous study, we detected chronotropic and inotropic changes in endogenous rhythm of the semi-isolated heart of ageing beetles. We also observed that stimulating impact of CCAP on the heart was age-dependant. CCAP application caused, depending on age, alternations in chronotropic and inotropic parameters of the heart contractions and the myocardium of the older beetles was less sensitive to the peptide.

The aim of the present study was searching for the coding sequence of the CCAP receptors in the genome of ageing *T. molitor* beetle and in longer term, the analysis of the expression of the genes in different tissues during ageing, especially in the heart. Based on the genom sequence of the most related beetle – *T. castaneum* we were able to determine the presence of two CCAP receptors in *T. molitor*. We also detected, for the first time, that both of the receptors are expressed in the heart of *T. molitor* beetle. Grant support: N N303 805640 (National Science Centre).

P27-82
A novel thiazolidinedione with low activity on differentiation of mouse preosteoblasts and preadipocytes

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Thiazolidinediones (TZDs) are insulin-sensitizing antidiabetic agents. Increased risk of bone fractures and weight gain are described as side effects which might be associated to an imbalance in osteoblast/adipocyte differentiation. In this work, the biological effects of GQ-177, a modified TZD, were explored on osteogenesis and adipogenesis and compared to that obtained with rosiglitazone. MC3T3-E1 preosteoblasts and 3T3-L1 preadipocytes were induced to differentiate with the appropriate induction media in the presence of TZDs. Effects on osteogenesis were evaluated by measuring matrix mineralization and alkaline phosphatase activity, while on adipogenesis were determined by lipid accumulation. Osteoblast and adipocyte markers and the Wnt/b-catenin pathway were also investigated by real time PCR and Western Blot. Unlike rosiglitazone, GQ-177 did not show inhibitory effects on matrix mineralization and alkaline phosphatase activity, increasing Wnt/b-catenin signaling and osteocalcin mRNA expression. However, GQ-177 decreased Runx2 and a1-collagen mRNA expression in preosteoblasts. Interestingly, GQ-177 showed no effect on b-catenin expression and lipid accumulation in preadipocytes besides to increase PPARg2, C/EBPa, aP2 and CD36 mRNA expression in a lower magnitude when compared to rosiglitazone. Altogether, our data indicate that GQ-177 would be a promising drug candidate with lower effects on cell differentiation without the side effects of inducing bone fractures and weight gain.
Financing: FAPESP, CNPq, CAPES.

P27-83
Mass spectrometry based multiplex BRCA gene mutations genotyping

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A novel multiplex mutation genotyping method was developed by utilizing ligase reaction and nicking amplification based on matrix assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS). In the first step of this method, ligase reaction was performed using two ligation probes that flanked the mutation site. The primary probe is designed to contain a complementary base of that of mutation site at its 5′ end and a primer annealing site for nicking amplification at

the 3′ end. The secondary probe is designed to have a nicking enzyme recognition site and a mass marker sequences at its 5′ end. Therefore, the primary and secondary ligation probes were linked by DNA ligase only in the case of mutant sample. The ligation product was then utilized as a template for nicking amplification reaction. The universal primer was annealed to the 3′ end of the ligation product and extended by DNA polymerase. As a result, nicking enzyme recognition sequences were formed and one DNA strand was released by the cleavage of nicking endonuclease. Since the cleaved short DNA segment is used as a mass marker, the amount of mass markers could be amplified by nicking enzyme amplification reaction. Finally, the mass marker was subjected to MALDI-TOF MS and the mass peak was detected only in the case of mutant samples. This strategy is very suitable for multiplex genotyping of target gene because the weight of the mass marker is variable. Using this strategy, we were successfully genotyping of 10 mutation sites in BRCA 1 gene in a single reaction tube.

P27-84
The effects of clinoptilolite-supported feeding on the iron level of tissues and blood in rats with iron overload

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Recently, the use of natural zeolites in animal nutrition has risen, mainly to improve their performance and health, and to protect against to heavy metal toxication. Several of zeolite applications take advantage of the adsorption and ion-exchange. That is why we investigated the effects of clinoptilolite (natural zeolite) from Turkey, Gordes in rats with iron overload. The rats were divided into four experimental groups which were control (C), clinoptilolite (Cli), iron (Fe), and clinoptilolite plus iron (Cli + Fe). The rats in groups of Fe and Cli + Fe received iron (Ferro III hydroxide polymaltose) by gavage at a dose of 250 mg/kg/day for 10 days, and also group Cli and Cli + Fe were fed a diet with 50% clinoptilolite for one month. The iron levels of tissue and serum from the rats were measured by the use of atomic absorption spectrometry. According to our experiment's results, we observed that the clinoptilolite decreased significantly the iron level in the stomach tissue. In contrast, the clinoptilolite increased the iron absorption to the small intestine tissue from lumen while it decreased the iron transportation into blood. The iron levels of clinoptilolite groups (Cli and Cli + Fe) were decreased in the tissues of heart, lung, liver, kidney and spleen with the various decreasing amounts due to the different amount of iron needs of each tissue in comparison with the group with iron overload (group Fe). The clinoptilolite can protect the body tissues against to iron toxicity by increasing the absorption of iron in the small intestine but the iron levels decreases in blood. We would like to thank to Rota Mining Corporation for clinoptilolite used in the study, and to Justenglish Language and TOEFL Center for their sponsorship to our study.

P27-85
Application of DNA adductome analysis to reporter gene mutation assay to understand chemical carcinogenesis

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DNA adductome analysis using liquid chromatography with tandem mass spectrometry (LC-MS/MS) allows comprehensive monitoring of chemical-specific DNA adducts. The development of transgenic rodents carrying reporter genes enabled examination of the *in vivo* genotoxicity of chemicals while also considering their biological behavior such as absorption, distribution, metabolism and excretion. Therefore, application of DNA adductome analysis to *gpt* delta rats could be a powerful tool for investigating the underlying mechanisms of chemical carcinogenesis. To examine the applicability of this assay, we carried out DNA adductome analysis and *gpt* and Spi$^-$ assays on the kidneys of *gpt* delta rats treated with madder color (MC) and its components, lucidin-3-*O*-primeveroside (LuP) and alizarin (Alz) in the diet for 8 weeks. In the DNA adductome analysis, spots indicating DNA adduct formation were commonly detected in MC- and LuP-treated rats, but not those treated with Alz. Two of these spots were identified as Lucidin (Luc)-N^2-dG and Luc-N^6-dA, respectively. Quantitative analysis using LC-ESI/MS/MS detected Luc-N^2-dG and Luc-N^6-dA at 21.0 ± 2.9 and 55.4 ± 3.9/10^9dG and 2.6 ± 0.4 and 3.9 ± 0.8/10^9dA in MC- and LuP-treated rats, respectively. *gpt* and Spi$^-$ mutation frequencies were significantly increased in the kidneys of rats treated with MC and LuP. Analysis of the *gpt* mutant spectra revealed that frequencies of AT-TA transversion and AT-GC transition mutations were also significantly increased in MC- and LuP-treated rats. Our new approach using *gpt* delta rats with DNA adductome analysis clearly demonstrated the major constituent responsible for MC carcinogenesis.

P27-86
Inhibition of glutaminyl cyclase attenuates cell migration modulated by monocyte chemoattractant proteins

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Glutaminyl cyclase (QC) catalyzes the formation of N-terminal pyroglutamate (pGlu) in peptides and proteins. pGlu formation in chemoattractants may participate in the regulation of macrophage activation and migration. However, a clear molecular mechanism for the regulation is lacking. This study examines the role of QC-mediated pGlu formation on monocyte chemoattractant proteins (MCPs) in inflammation. We demonstrated *in vitro* the pGlu formation on MCPs by QC using mass spectrometry. A potent QC inhibitor, PBD150, significantly reduced the N-terminal uncyclized MCPs precursor (preMCPs)-stimulated monocyte migration, whereas pGlu-containing MCPs (pMCPs)-induced cell migration was unaffected. QC siRNA revealed a similar inhibitory effect. Lastly, we demonstrated that inhibiting QC can attenuate cell migration by LPS. These results strongly suggest that QC-catalyzed N-terminal pGlu formation of MCPs is required for monocyte migration, and provide new insights into the role of QC in the inflammation process. Our results also suggest that QC could be a drug target for some inflammatory disorders.

P27-87
Structural elucidation of mixtures of saponins from corms of Crocus sativus combining NMR, HPLC-ESI-MS and GC-MS techniques

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Various compounds derived from pathogens, minerals or plants, possess pro-inflammatory properties which allow them to act as adjuvants and contribute to the induction of an effective immune response. CS5 is a HPLC fraction purified from the external part of saffron corms, which was efficiently used as adjuvants for protein-based vaccines formulations. The main components of this fraction were two triterpenoid saponins, Azafrine 1 and Azafrine 2, which represent almost 70% (w/w) of CS5 content. The rest of 30% (w/w) of CS5 fraction, which were also saponins, have been fractionated with a simple HPLC step on a C18 column to be tentatively elucidated by comparison with Azafrine1 and Azafrine 2, through NMR, HPLC-ESI-MS and GC-MS techniques. These analyses have been carried out without a complete purification process to avoid costs and time consuming. The diversity between the elucidated saponins is mainly due to different degree of glycosylation of echinocystic acid or similar glycosylation pattern with oleanolid acid as sapogenin.

P27-88
Characterization of the transmembrane domain of the yeast essential protein, Rot1

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Membrane proteins are important fraction of proteome of living beings. Rot1 is an essential membrane protein from budding yeast *Saccharomyces cerevisiae* related to cell wall biosynthesis, actin cytoskeleton dynamics and protein folding. Rot1 is translocated to endoplasmic reticulum (ER) by an SRP-independent post-translational mechanism and remains anchored to the nuclear envelope ER membrane by its C-terminal transmembrane domain. Previously, we have shown that this domain is necessary, apart from the anchor, to the correct protein function, as yeast cells with truncated forms at the C-terminal end of Rot1 are unviable. To identify the most critical segment of the transmembrane helix we proceed to scan this domain by Ala insertion scanning mutagenesis, finding that amino acids 236–250 are the residues involved in Rot1 normal activity. Further, we focused on analyzing the key residues for protein function by punctual mutations in this protein segment. Analysis of mutants showed that Ser250 is required to sustain cell viability. Interestingly, the substitution of Ser250 by threonine does not affect protein function, suggesting that the single residue Ser250 is involved helix-helix transmembrane association by its hydroxyl group.

P27-89
Illness pattern and the relationship between the prevalence of malaria and other infections in Niger Delta University Bayelsa state, Nigerria

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The illness pattern and the relationship between malaria and other infections were examined in the present work – base-line information prior to a meaningful healthcare policy formulation for a progressive economic development. A period of 5 years (2007–2011) illness pattern was retrospectively obtained from the University medical centre, while questionnaires addressing the various health-related activities were administered to 358 volunteer subjects – students and staff of the institution. Biochemical parameters in the various disease conditions and the means of frequencies of disease occurrence were compared using statistical package for social sciences (SPSS). Results of mean comparison showed that the mean of frequencies of malaria infection was significantly increasing ($p < 0.05$). On the other hand, differences in the means of serum elements and other biochemical parameters measured in the various disease states were not statistically significant ($p > 0.05$). Malaria infection was the most prevalent illness with the highest frequency occurring around the month of April, accounting for 63.82–67.10% of the illnesses compared. This was followed by Upper Respiratory Tract Infection (URTI), peptic ulcer, and candidiasis, in that order. The relationship between the illness types showed that a positive correlation existed between malaria and typhoid, and malaria and URTI, ($p < 0.05$). From the results of questionnaire analysis, 70.89% of the male students' group admitted having a frequency of one to twelve attacks of malaria infection per annum with 43.67% of them who claimed to have suffered previous typhoid infections. As a result, 25.32% were absent from lectures for a period of 1–30 days per annum. These results showed that malaria infection was on the increase and apart from impacting negatively on students' performance, could represent a huge source of financial drain on a developing economy.

Keywords: illness pattern, malaria, typhoid fever, infections.

P27-90
Iron and ferritin removal from serum of beta thalassemia patients with iron overload by synthetic zeolites

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Beta thalassemia is a genetic defect which causes incorrect synthesis of one of the hemoglobin's globin chains. Beta thalassemia patients require medical treatment including blood transfusion regimen and iron chelation. However, in some patients iron level in blood serum does not decrease even though the iron chelating therapy is applied, thus the patients have overloaded iron. Heavy metals such as iron are not biodegradable so they accumulate in

organisms, and cause some disorders. Therefore, they need an additional therapy to withdraw the iron from body for a qualified life. Zeolites are naturally occurring silicate minerals, while synthetic ones are also being produced for specific applications. The zeolite's structure is negatively charged and attracts cations that come to reside. The ion-exchange process is reversible, and this allows the adsorption of ions and molecules as well as making zeolites chemical sieves which help to remove the toxins. In this project, we studied two different zeolite types to understand whether they adsorb ferritin and iron *in vitro*. In addition,we investigated the other serum proteins, Cu and Zn levels that have a regulative relationship with iron. We utilized 341NHA(N-zeolite) and390HUA(H-zeolite) from HSZ®-300 Series (USY Zeolite) by TOSOH Corporation. H-zeolite decreased the level of iron (II), copper (II), zinc ions, ferritin and total protein levels in blood serum by 27.2%, 24.8%, 42.3%, 14.8% and 19.4% respectively while N-zeolite decreased the level of iron (II), copper (II), zinc ions, ferritin and total protein levels by 32.2%, 31.1%, 12.5%, 32.1% and 26.9%, respectively. As a result, synthetic zeolites had selectivity for different cations based upon carrying H+ or NH4+ as a structural cation. Remarkable properties of zeolites will be utilized in the near future for the environmental and medical applications, and our research is a preliminary study to comprehend the usage possibility of zeolites in apheresis and hemofiltration systems. We would like to thank to Justenglish Language Center for their sponsorship.

P27-91
Studies on the application of thermoluminescence, electron spin resonance, and GC/MS methods for detection of irradiation in foods

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The irradiation to foods is well-known for one of the most useful method to inactivate pathogenic microorganisms and to improve the storage efficiency. While Codex Alimentarius Commission (CAC) has adopted general standards and the methods for the detection of irradiated foods, each country developed domestic guidelines on admitted food items and applicable detection methods to guarantee the safety of irradiated foods to its people's health. The detection methods like Thermoluminescence (TL), Electron Spin Resonance (ESR), Photostimulated luminescence (PSL) and GC/MS methods are primarily used for identifying irradiated foods but they are not utilized broadly to various foods yet. So this study was conducted for the purpose to investigate whether TL, ESR and GC/MS detection methods could be applied to identify imported food items which have not been allowed to be irradiated in Korea. We are planning to analyze approximately 65 imported foods items including dried tropical fruits, dried mushrooms, wheat flour and seeds. Preliminary study results are obtained for 20 items including dried fig, flour and curcumin. It was shown that TL detection method could be applied to all the seven tested foods suggesting they contained enough quantity of minerals. ESR signal intensities of three irradiated foods, dried fig, flour and curcumin were not symmetrical compared to the ones for non-irradiated foods. In particular, the radiation signal was not detected in wheat flour at all. We are currently testing continuously all 65 foods to identify the applicability of three test methods for individual item. The interim result will be reported at the meeting.

P27-92
Effects of luteolin on liver, kidney and brain in PTZ-induced seizures: involvement of metalloproteinases and NOS activities

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Flavonoids are important group of recognized antioxidants in plants. Luteolin (LUT) is a natural flavonoid in the plant kingdom. This study was aimed to investigate the effects of the LUT in the liver, kidney, brain of pentylenetetrazol (PTZ)-induced seizure and the relationship between nitric oxide synthases (iNOS, eNOS) and matrix metalloproteinases (MMP2, MMP9). LUT (10 mg/kg) was given intraperitoneally during two weeks prior to seizure induction. Single dose PTZ 80 mg/kg i.p. administered and seizure were observed and evaluated regard to latency, frequency and stage for one hour. Seizure frequency after PTZ administration was significantly decreased in LUT pretreated rats ($p < 0.05$). An increase of immunhistochemical reactions of iNOS, MMP2, but a decrease of eNOS activity were observed in rat hippocampus and peripheral tissues during the PTZ induced seizures. LUT pretreatment reversed the iNOS, MMP2 activity to the control levels and significantly increased the eNOS activity ($p < 0.001$). LUT seems to have an effective role in reducing the seizure frequency and protective role on peripheral organ injury in animal model of seizure. The protective effect of LUT in seizures and the seizure induced peripheral tissue damage warrant further investigations.

P27-93
New erm gene in *Bacillus halodurans* C-125, alkaliphilic bacterium

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Electronically annotated *erm* gene from *Bacillus halodurans* C-125, alkaliphilic bacterium was cloned and characterized. The deduced amino acid sequence of new *erm* gene showed 66% and 61% of sequence identity with ErmD and Erm (34) respectively implying that this new *erm* gene is of novel class. While *E. coli* expressing ErmN (monomethylase) was susceptible to erythromycin and tylosin on agar plate, *E. coli* harboring new *erm* gene showed resistance to both antibiotics as observed in *E. coli* expressing ErmC (dimethylase), suggesting that this new Erm protein is a dimethyltransferase. To verify this, total rRNA was isolated from *E. coli* cells expressing the new Erm protein and analyzed for its methylated state at the specific adenine residue, A2058 with reverse transcription using primer complementary to the sequence a few nucleotides apart from A2058. Whereas reverse transcriptase could pass through N^6 monomethylated adenine, dimethylated adenine does not allow the reverse transcriptase to move beyond it. Extension with reverse transcriptase stopped at A2058, resulting in a clear autoradiogram band corresponding to A2059. So, it was concluded that it is one of the real dimethylating *erm* genes and was tentatively named ErmK? ErmK? exhibited very low methylating activity compared to the ErmC. This might represent one of characteristics of proteins that are present in alkaliphile.

P27-94
The cytoplasmic cyclophilin negatively affects bacterial swarming motility and biofilm formation ability

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Due to its cyclic side chain, proline can adopt both *cis* and *trans* conformations about its peptide bond, creating distinct and interconvertible backbone structures. Peptidyl-prolyl *cis*/*trans* isomerases (PPIases, EC: 5.2.1.8), speeding this interconversion control the duration and amplitude of a variety of cellular processes such as cell-cycle, protein quality assessment and turnover and various cancers while they can also act on polypeptides as folding helper enzymes. Bacterial PPIases although appear to be non essential for growth under laboratory conditions they have significant roles in survival in environmental and pathogenic niches. In the present study we show that *Escherichia coli* cytoplasmic cyclophilin negatively affects specific forms of bacterial social behavior such as swarming motility and biofilm formation. We phenotypically characterized deletion and over-expression strains under swarming/swimming and biofilm conditions and by RT-qPCR we checked whether hypermotility is mediated through changes in flagella biosynthesis. Since PPIases constitute three convergently evolved gene families, we clarified the functional redundancy of the cytoplasmic cyclophilin with the rest members of all three families under these conditions. In complementation studies we over-expressed each PPIase in the cytoplasmic cyclophilin deletion mutant while we also measured the expression of each PPIase gene in this mutant using RT-qPCR. Further, in an effort to understand whether the observed phenotypes are caused by alterations in various cyclophilin prey proteins levels we performed complementation analyses with various prey proteins while we also measured their gene expression levels in the cyclophilin deletion strain grown under these conditions.

P27-95
Tissue localization of intracellular gelatinolytic activity

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The matrix metalloproteases (MMPs) are a family of 23 enzymes. MMP-2 and MMP-9 are structurally very similar, and make up the gelatinase group. MMP-2 is the most widely expressed of all MMPs, and is constitutively produced in most tissues and cells. In addition to gelatin, MMP-2 is able to degrade a number of other matrix and non-matrix substrates. MMP-9 is able to process many of the same ECM substrates as MMP-2, but is normally only expressed in a few cell types. MMP-2 and MMP-9 contain a signal sequence which directs them for secretion and are extracellularly working enzymes. Nevertheless, an increasing number of papers report that MMPs may also have intracellular functions, but the extent of this activity during normal homeostasis is not known. Due to the emerging knowledge of intracellular substrates and functions, the present study was conducted to assess possible intracellular MMP-2 and MMP-9 activity in a number of normal tissues by combining immunohistochemical staining for the enzymes and *in situ* gelatin zymography, a method used to localize and quantify gelatinolytic activity in tissue sections. We found that a number of different tissues show intracellular gelatinolytic activity that co-localized with MMP-2 staining. In many cell types the level of MMP-2 staining correlated with degree of gelatinolytic activity, and the activity was markedly inhibited by addition of the metalloprotease inhibitor EDTA. In contrast, MMP-9 staining was hardly detected in the tissues analyzed. This indicates that a number of cell types may have intracellularly active MMP-2, though the functions of this activity is not yet known. MMP-2 might have important roles inside cells during normal homeostasis, and this should be taken into account when new MMP-inhibitors are designed for treatment of diseases such as cancer.

P27-96
On the origin of cancer stem cells

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Tumors are characterized by their cellular heterogeneity due to the co-existence of different cellular sub-populations, whose hierarchic organization in certain cancers lead to the hypothesis that the target cells of transforming mutations are stem cells. However, in other tumors, restricted progenitors or even differentiated cells may be the cell of origin. Cancer stem cells (CSCs) are consequently, stem-like cells with self-renewal and multipotent differentiation characteristics which can originate all cell types found in a tumor (1). Unexpectedly, while attempting to understand the mechanisms underlying hexavalent chromium induced lung cancer, we demonstrated that CSCs could be obtained by dedifferentiation of the malignant bronchial epithelial cells DRenG2 and DDRenG2 and/or their precursor RenG2. The present work characterizes the proliferation rate of RenG2, DRenG2 and DDRenG2, and their normal precursor namely BEAS-2B and assesses the cytogenetic evolution from BEAS-2B to DDRenG2. The cytogenetic analysis of the more malignant and more proliferative DRenG2 and DDRenG2 revealed common structural differences relative to progenitor BEAS-2B cells namely $7p^-$ and t(16:17). However, DRenG2 revealed the predominance of $7q^-$ and $iso9q^+$ while DDRenG2 t(7:14). In contrast to the less proliferative BEAS-2B, and similarly to RenG2 both DRenG2 and DDRenG2 predominant ploidy was 75/76 chromosomes. In conclusion, the more malignant DRenG2 and DDRenG2 cell lines are highly proliferative and bear characteristic chromosome aberrations that may possibly identify specific gene alterations involved in the process of dedifferentiation to CSCs.

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P27-97
Biodegradation of 2,4,6-trichlorophenol catalyzed by acetosyringone-laccase mediator system

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Laccase is a multicopper oxidoreductase (EC 1.10.3.2) which catalyses the oxidation of a wide range of substrates by the 4 e^-

reduction of O_2 to H_2O by using copper centres of three different types. The high oxidation potential allows the broad applications in biotechnology that laccase possesses. The used of mediators with laccase (LMS) increase the numerous of processes in which lacasse is applied. The search of efficiency, cheap and eco-friendly mediators is increasing the last years, for that natural mediators are the most appropriate ones, being acetosyringone (ASG) the natural phenolic compound studied. 2,4,6-Trichlorophenol (TCP) is a pesticide, antiseptic and wood preservative, classified as environmental pollutant and probable human carcinogen by the USEPA. The airborne fungi can convert TCP into 2,4,6-trichloroanisole (TCA), chief cause of cork taint from polluted cork stoppers to wines. The aim of this work is report on the ability of commercial laccase (*Trametes villosa*, Novozymes) for the biodegradation of TCP. Parameters as buffer pH value and the concentration of all species involved in the reaction were optimized and a kinetic study with its corresponding mechanism of reaction are proposed. The best assay conditions for the biodegradation in 12 min of 1.05 mM TCP are 300 nM LAC, 350 μM ASG, and 50 mM pH 4.0 at 25°C. The kinetic analysis of a reaction mechanism proposed for the enzymatic system, agree with the experimental results.This work has been partially supported by grants from several Spanish organizations. Projects BIO2009-12956 (MICINN, Madrid) and 08856/PI/08 (Fundación Seneca, CARM, Murcia). Predoctoral fellowship JMR BES-2007-16208 (FPI, MICINN, Madrid) and MP 09378/FPI/08 (Fundación Seneca, CARM, Murcia).

P27-98
Senescent bronchial fibroblasts drive bronchial epithelial cells metaplasic transformation following exposure to hexavalent chromium

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Cellular senescence, associated with aging and/or stress insults, can prevent neoplastic transformation. However, accumulated evidence showed that senescent cells can stimulate malignant phenotypes in nearby cells by secreting protumorigenic factors that stimulate epithelial cells proliferation and disrupt epithelial differentiation [1]. Senescent stromal cells also confer invasive and migratory phenotypes by promoting EMT [2].

Here we report that sub-cytotoxic doses (0.25 and 0.5 μM) of Cr(VI), a human lung carcinogen known to induce squamous cell carcinomas [3], induced the senescence of normal human bronchial fibroblasts (E2A) which stimulated the proliferation and a striking change in the phenotype of human bronchial epithelial cells (BEAS-2B). In fact, co-cultivating senescent E2A with BEAS-2B cells in presence of 0.25 μM stimulated BEAS-2B cells to acquire a basal cell phenotype (MNF^+, $Vimentin^+$) with large round nuclei and ted-pole cytoplasm characteristic of epidermoid metaplasia. Moreover, co-cultures exposed to 0.5 μM Cr(VI) lead BEAS-2B cells to acquire a cuboid morphology with many mitotic figures and activated nuclei with visible nucleoli, as well as fusiform shape associated with EMT phenotypic switch. Additionally, senescent E2A cells co-cultured with Cr(VI)-treated BEAS-2B cells acquired mesenchymal features i.e., $Vimentin^+$ and α-SMA^+ large stellate cells with heterogeneous size enlarged nuclei, particularly abundant for 0.5 μM Cr(VI) which also lead to the appearance of crisscrossing. In sum, our results suggest that in presence of Cr(VI) the crosstalk between senescent fibroblasts and epithelial cells, mediated by secreted factors by both cell types, induced premalignant features in BEAS-2B cells and a more undifferentiated phenotype in fibroblasts.

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P27-99
Sensitivity of dwarf elder (*Sambucus ebulus* L.) fruit lectins to a simulated gastric fluid

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In recent years a close relationship between the presence of allergens in pollen and foods derived from plants and the allergic response has been suggested. Some authors have reported that low gastric digestibility is not an absolute criterion for food protein allergens [1]. Some lectins from *Sambucus* spp. share amino acid sequences with the pollen Sam n1 allergen [2]. The lectins ebulin f and SELfd from the early stages of growth [3] were isolated and subjected to analysis by MALDI-TOF mass spectrometry, tryptic peptide fingerprinting, molecular characterization and pepsin digestibility. Both molecular masses and amino acid sequence analysis indicated that Sam n1 fits better with a monomeric lectin like SELld (Mr 34,2) found in shoots of dwarf elder [4]. Ebulin f toxicity to mice was higher intraperitoneally than orally at the same dose (5 mg/kg body weight). In contrast SELfd at the same dose lacks of apparent toxicity. Ebulin f, but not SELfd, undergoes extensive pepsin proteolysis, which could explain the differences in toxicity. The present study supports our hypothesis that the Sam n1 allergen could be a sequence-related monomeric lectin like SELlm present in shoots of *S. ebulus* rather than ebulin.

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P27r-100
Green tea polyphenols enhance the toxicity of nigrin in mice

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A number of plant lectins inhibit protein synthesis through the irreversible inactivation of ribosomes. They are known as ribosome-inactivating proteins (RIPs). Among the two chains RIPs (A-S-S-B) stand out nigrin b from bark elderberry (*Sambucus nigra* L.) [1]. Nigrin b has been used for anticancer therapy as immunotoxin [3]. The A chain has N-glycosidase activity on the 28S rRNA and the B chain is a D-galactose-binding lectin. It has been shown that the intravenous administration of large doses of nigrin b to mice is toxic with a LD_{50} of 12 mg/kg body weight [2]. We investigated the effects of the intraperitoneal administration of nigrin b and the potential interactive action of the green tea polyphenols given orally. Polyphenols display anti-oxidant effects at low doses and pro-oxidant at large doses. The pro-oxidant effects have been described to cause apoptosis. In this study, nigrin b was administered either at 10 or 16 mg/kg body weight together with a standard preparation of green tea polyphenols (Polyphenon E) and Kaplan-Meier plots of survival, weight changes and histological analysis were carried out in these animals. Our results indicated that Polyphenon E administration enhances nigrin b toxicity both at 10 and 16 mg/kg body weight. The mechanism of action of these effects seems to be through the synergy of the apoptopic action of both nigrin b and polyphenols.

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P27-101
Toxicity to mice of the type 2 ribosome-inactivating protein from dwarf elder (*Sambucus ebulus* L.) fruit

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Ribosome-inactivating protein (RIP) triggers the hydrolysis of the N-glycosidic bond between A_{4324} and the phosphate backbone of the 28S rRNA [1]. Among them are ricin, abrin, volkensin, ebulin and nigrin. Ebulin f is a 56 kD RIP with two chains (A-S-S-B) present in dwarf elder (*Sambucus ebulus* L.) fruits [2]. The A (26 kD) chain has N-glycosidase activity and the B chain (30 kD) is a D-galactose specific lectin. Ebulin f has 1.5×10^{-4} times less toxicity for HeLa cells than ricin [1]. The reason seems to be related with a change in the affinity for galactosides of the sugar-binding 2γ-subdomain of the B chain [3]. Nonetheless, we found that the intraperitoneal administration of large amount of ebulin f (5 mg/kg body weight) to mice displays a powerful intestinal toxicity without apparent deleterious effects on other tissues. The toxicity is observed first in the intestinal crypts which loss their cellular structure and after in the ablation of villi. Sublethal doses of ebulin f (2.5 mg/kg body weight) promote similar but less harmful effects. The toxicity is paralleled by an important decrease of the body weight which is reversible at sub-lethal doses. Our results suggest that the toxic effects of dwarf elder fruits could be due to ebulin f.

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P27-102
Guidance of dermal cells distribution by laser-structured ormosils

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Several dermal substitutes for skin grafting are now commercially available, although their performance still needs improvement. Most artificial dermises have a lower take rate than autologous grafts and require more time for sufficient vascular in growth to overlay the skin graft. Herein we characterize new two-dimensional scaffolds for tissue-engineering applications, which were fabricated by two-photon polymerization (2PP) of hybrid methacrylates based on silane derivates (ormosils). They have been previously considered for applications in electronics, microtechnology, corrosion resistant coatings, dentistry, and biomedical implants. The presence of both inorganic chains and organic groups can result in the appearance of new thermal and chemical properties. In this study, we showed that the polymeric structures with controlled architectures produced *via* 2PP could be used as scaffolds for the *in vitro* culture and proliferation of human dermal fibroblasts. Fluorescence microscopy revealed that the fibroblasts' orientation was guided by the scaffold geometry, consisting of ormosils lines or grids. This 'dermal equivalent' was investigated for its ability to accommodate epidermal cells. To evaluate this interaction, two experimental approaches were hence used: (i) fibroblast–melanocyte co-cultures; and (ii) fibroblast–keratinocyte organotypic cultures. During their growth on ormosil scaffolds, productive interaction of fibroblasts with both epidermal cell types was found. Moreover, this pseudo-dermis was shown to support the growth of keratinocytes for up to 8 days after their seeding.

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P27-103
Determination of serum micrornas for early detection of gastric cancer

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Gastric cancer (GC) is the fourth most common malignancy and the second leading cause of cancer death in both gender over the world. The clinical outcome of GC has gradually improved, but the survival rate of patients with advanced GC is still disappointing. Understanding the molecular structure of GC carcinogenesis might identify new diagnostic and therapeutic strategies for this disease. Thus, early detection of GC is a key measure to reduce the mortality and improve the prognosis of GC. There have recently been several reports that miRNAs circulate in highly stable, cell-free forms in blood. Because serum and plasma miRNAs are relatively easy to access, circulating miRNAs also have great potential to serve as non-invasive biomarkers. MiRNAs are small 22 nt, endogenous, single-stranded, non-coding regulatory RNA molecules which regulate the expression of up to 30% of human genes, either by inhibiting mRNA translation or inducing its degradation. Recent evidence has demonstrated that miRNAs regulate leading cellular processes involved in tumor biology, including cell proliferation, differentiation, programmed cell death and metastasis. Several studies of aberrant expression of specific miRNAs have been reported in GC. In GC patients, plasma miR-21 and miR-106b were reported to be potential diagnostic biomarkers for GC and serum miRNAs (miR-1, miR-20a, miR-27a, miR-34, and miR-423-5p) could serve as fingerprints for GC diagnosis. Although a number of miRNAs associated with GC have been identified, the underlying mechanism of these miRNAs in tumorigenesis and tumor progression remains to be investigated. In this study we tried to identify the potential of serum miRNAs as biomarkers for early detection of GC patients. Blood samples which drawn in EDTA tubes were accomplished by centrifugation at 4000 rpm for 15 min for serum separation. The supernatant of serum was recovered and stored at -80°C until analysis. RNA was isolated using the High Pure miRNA Isolation Kit (Roche) following the manufacturer's protocol. cDNA and preamplification protocols were obtained from the isolated plasma miRNAs. The BioMark™ 96.96 Dynamic Array (Fluidigm Corporation) for real-time qPCR was used to simultaneously quantite the expression of 768 miRNAs. All statistical analyses were performed using the Biogazelle's qbase PLUS 2.0 software. Statistical analysis of 768 miRNA between gastric cancer and control groups were compared with the Mann–Whitney *U*-test. $p < 0.05$ was considered statistically significant. In this study, among 768 miRNAs that we analyzed only miR-195-5p was significantly ($p < 0.05$, fold changes = 13.3) down-regulated in GC patients compared with control.

P27-104
Structure-function relationships of lung surfactant membranes and films under thermal stress

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Pulmonary surfactant (PS) is a lipo-protein mixture, which lines the entire alveolar surface, and is primarily involved in reducing interfacial surface tension and thereby stabilising the lung, lowering the work of breathing. Heterothermic mammals experience intermittent periods of metabolic depression, including torpor or hibernation associated with a substantially reduced living temperature. They have an unusual surfactant composition with large amounts of fluid lipids including unsaturated phospholipids and neutral lipids like cholesterol. Specifically, palmitoyl palmitoleoyl phosphatidylcholine (PPPC or PC16:0/16:16–10% in homeothermic mammals) is present abundantly (20–30%) in heterotherms. However, the role of PPPC is unclear. It is also unclear how the biochemical adaptations alter surfactant function under different thermal conditions and how such surfactants are able to attain low surface tension at low temperatures. We explored the thermotropic and phase segregation properties of PS membranes and films from two heterotherms, fat-tailed dunnarts (*Sminthopsis crassicaudata*) and Gould's wattled bats (*Chalinolobus gouldii*). Surfactant membranes of torpid versus warm-active animals possessed lower enthalpy, indicating increased fluidity. However, fluorescence spectroscopy and anisotropy showed a cooling-induced phase packing, such that surfactant from torpid and warm-active animals possessed a similarly dehydrated, ordered phase. Epifluorescence microscopy revealed changes in compression-induced lipid packing between surfactant films of warm-active and torpid animals, leading to structural differences in domain morphology and distribution. Pressure-area isotherms suggested that PPPC, unlike dipalmitoyl PC (DPPC) or palmitoyl oleoyl PC, forms tighter phases with cholesterol at 20°C. PPPC plus cholesterol may complement DPPC phases in forming stable PS films upon expiration.

P27-105
The stability estimation for korean national biological reference standards: Bordetella Pertussis and Anti-Pertussis

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Biological products include a wide range of products such as vaccines, blood-derived products, toxins, recombinant therapeutic proteins, cell therapy products, and gene therapy products. Reference standard is a biological material one or more of whose property values (potency, amount, etc.) are sufficiently well established to be used in assessing a measurement method or assigning values to other biological materials. According to WHO recommendation, we as national authority, obtain minimum amount of standards from WHO international laboratories and establish our own national biological reference standards (NBRSs). A high degree of stability is one of essential requirements for NBRSs.

In this study, stability tested for Bordetella Pertussis Vaccine and Anti-Pertussis NBRSs were performed. The mouse body weight gain test (MWGT), the leukocytosis-promoting test (LPT), and the histamine sensitization test (HIST) are done for the testing of the specific toxicity of pertussis vaccines. For the Anti-Pertussis, identification test (Ouchterlony test) were performed. The trend analysis in lymphocytosispromoting units and histamine-sensitizing units were stable but body weight-decreasing units (BWDU) were decreased. Identification test on Anti-Pertussis showed clear precipitin lines.

Taken together, it has been proposed as follows; Bordetella Pertussis vaccines and Anti-Pertussis which are made by KFDA as a national biological reference standard, showed stable potency.

P27-106
The influence of various agents disturbing cell division process on lipid domains morphology in *Escherichia coli* filamentous cells

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Bacterial cell membrane is known as heterogeneous and dynamic structure consisting of domains and/or lipid rafts, that allow to compartmentalize and regulate biological processes. Also it can be an important element in the bacterial response to stress inducing conditions, such as the presence of antibiotics. Since this stress response often leads to achieving of persistence to antibiotic treatment by bacterial cells, it seems interesting to check the influence of various antibacterial agents on the cell membrane domains morphology. It is well known that different antibacterial agents induce formation of filamentous cells regardless of their mode of action. Our previous results have shown distinct membrane domains perturbation or/and unusual distribution in filamentous uropathogenic *Escherichia coli* cells induced after the ciprofloxacin treatment – antibiotic which is known to act at the DNA level. In the present study we explore the changes in lipid domains morphology in relation to nucleoid location in *E. coli* cells treated with subinhibitory concentrations of ciprofloxacin and other cell division process disturbing agents: chlorpromazine (penicillin-binding-proteins inhibitor), cinnamaldehyde and berberine (FtsZ ring formation disturbing agents). Despite the fact that these compounds act at different stages of cell division process we noticed that all of them cause the formation of filamentous cells with similar pattern of anionic lipids distribution and DNA position. It may suggest, that membrane lipid domains may be involved at different stages during the cell division.

P27-107
An electrochemical washing-free DNA detection utilizing gold nanoparticle-labeled signaling probe

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A one-step electrochemical method for the detection of DNA based on transport control of the signaling probe labeled with gold nanoparticles has been developed. On the gold matrix immobilized with capture probe, both unpurified target DNA sample amplified from the hemagglutinin (HA) gene of the H1N1 virus and AuNP-tagged signaling probe are introduced. The target DNA induces sandwich DNA hybridization with both the capture probe immobilized on the gold matrix and signaling probe labeled with gold nanoparticle leading to the immobilization of the AuNP on the gold matrix. In this state, the square wave anodic stripping voltammetry (SWASV) of the gold nanoparticle on another bismuth-coated glassy carbon (GC) electrode show no current signal because there is no AuNP transported to the GC electrode surface. When there is no target DNA in the sample, on the other hand, freely diffusible AuNP-labeled signaling probe can transport to the GC electrode and generate electrochemical current signal. Finally, our system shows the reliable signal change according to the concentration of target DNA without any DNA purification or secondary reaction step.

P27-108
Reaction of human dermal fibroblasts to mitoxantrone treatment

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Aim: The aim of our study was to determine the response of human dermal fibroblasts (HDFs) to DNA-damaging chemotherapeutic agent mitoxantrone (MTX). We focused on the effects of MTX on proliferation, viability, cell cycle changes and reaction of key proteins which play important role in cell proliferation, senescence and apoptosis of HDFs.
Methods: Proliferation of MTX-affected HDFs was detected by Z2 Counter and viability by Vi-Cell XR using Trypan blue exclusion staining. Cell cycle changes were analyzed by flow cytometry. Protein levels at different time and concentration were evaluated by Western blotting. The activity of β-galactosidase was detected by Senescence β-galactosidase Staining Kit and γH2AX foci by immunocytochemistry. Induction of apoptosis was determined by monitoring of caspases activity by Caspase-Glo Assays.
Results: Our results showed typical senescent phenotype of MTX-treated HDFs. Proliferation of affected HDFs was completely inhibited and viability remained stable during 6 days. We observed enhanced activity of senescence-associated β-galactosidase, increased expression of $p16^{INK4a}$ and persisting DSBs-associated γH2AX foci which occurred 9 days after exposition to MTX. Moreover MTX provoked an increase in p53 and its phosphorylation of serine 15 as well as the expression of $p21^{WAF1/Cip1}$. Higher concentrations of MTX triggered caspase-mediated apoptosis.
Conclusion: Our data indicate that HDFs treated with clinical relevant concentrations of MTX underwent premature senescence, which was proved by increased activity of senescence-associated β-galactosidase, persisting γH2AX foci and upregulation of p16.
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P27-109
Characterisation of an atypical outer membrane protein targeting pathway in the Gram negative bacteria *Dickeya dadantii*

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Cell envelope of Gram negative bacteria consists in two membranes separated by an hydrophilic space called periplasm. As

other outer membrane constituents, proteins are synthesized in the cytoplasm and must cross inner membrane and periplasm before being inserted in the outer membrane. In *Escherichia coli,* the vast majority of outer membrane proteins consists of both β-barrel proteins and lipoproteins. In these bacteria, periplasmic chaperones SurA, Skp and DegP work together with the β-Barrel-Assembly Machinery (Bam) to target and insert β-barrel proteins in the outer membrane. In addition, the periplasmic chaperone LolA and the outer membrane lipoprotein LolB are respectively responsible for lipoprotein targeting and insertion in the outer membrane.

Recent work in the Gram negative phytopathogen *Dickey dadantii* has shown the existence of a third kind of outer membrane protein. The pectin lyase homolog, PnlH is anchored in the outer membrane by an uncleaved Tat signal sequence and does not share any structural characteristics with β-barrel proteins or lipoproteins. Moreover, when expressed in *Escherichia coli*, PnlH is found in the outer membrane. These observations indicate that a third outer membrane targeting pathway could exist in *Dickeya dadantii* and *Escherichia coli*.

In this work, we aimed to find out how PnlH is targeted and inserted in the outer membrane of *Dickeya dadantii*. Surprisingly, our results indicate that PnlH could use some components of the β-barrel proteins targeting pathway to reach the outer membrane and being inserted in it. Moreover we have been able to identify a small region of PnlH signal sequence that bears the information necessary for the targeting to the outer membrane.

P27-110
The surface EMG changes with aerobic-anaerobic transition intensity at incremental exercise in soccer players

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In physiological evaluations it has been postulated that Electromyographic Threshold (EMGT) may be used as a method to alternatively determine anaerobic threshold (AT). The correlation has been shown between the exercise intensity (AT2) that corresponds to the transition phase (AT2) expressing the beginning of the compensatory mechanism and EMGT exercise intensity. It is still controversial whether the EMG threshold value can be used as a valid method. The aim of this study, in control and soccer player groups, is to determine exercise intensities between the AT2 and EMGT, and to argue the utilization possibility of EMGT as an alternative noninvasive method.

In this study 16 male subjects performed the incremental exercise test to exhaustion on an electronically-braked cycle ergometer (workload starts with 5 min warm up at 50 watts and exercise load was increased by 25 watts every 2 min) to measure and evaluate performance parameters, electromyographic signals. During the test, respiratuar gas exchange data and Raw-sEMG signals were collected to determine Ventilation (VE), Heart Rate (HR), Respiratuar Exchange Ratio (RER), VO2, VE/VO2, VE/VCO2, Rating of Perceived Exertion (RPE), Anaerobic Threshold2 (AT2), Rms- EMG. Rms-EMGT was determined by Linear Regression Analysis.The results were compared statistically evaluated. SPSS 15.0 was used for statistical analysis.

The aerobic capacities of the soccer players were found significantly improved compared to the sedentary participants ($p < 0.001$). No significant relation was found between AT2 and Rms-EMGT of four seperate muscles. In conclusion, our findings suggest that Rms-EMG, may not be used a valid, noninvasive method for Anaerobic Threshold determination.

P27-111
Microbial encapsulation in monodisperse hydrogel microspheres enables fast and sensitive phenotypic analyses using flow cytometers

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Detection and characterization of microorganisms by classical procedures usually involves culture during more than 20 generations in order to achieve the formation of macrocolonies on solid media. This can be impractical when fast analysis of growth is required or when the organisms are not cultivable. To overcome these problems, new technologies based on single-cell encapsulation have been developed. Thereby, microencapsulation allows the detection of microbial growth facilitating the isolation, the analysis and the classification of microscopic colonies by monitoring their development from encapsulated individual cells. Microbial proliferation inside the microcapsules can be detected using flow cytometry without requiring fluorescent labelling provided that the population of microparticles exhibits appropriate optical and mechanical properties and is monodisperse in size and shape.

With this results we show the successful analysis of *Saccharomyces Cerevisiae* colonies using a Cellena® Flow Focusing® microencapsulator combining with flow cytometry. Moreover, we demonstrate the capacity of select a specific population of cells from a total one using the COPAS SELECT flow cytometer sorting system (Union Biometrica).

In addition, these technologies let us to encapsule bacteria and human stem cells and can be used for a variety of applications: from characterizing secreted enzymes to detection of thermosensitive mutants.

Alginate particle sizes were reproducibly selected from < 100 μm to over 600 μm, by just replacing the disposable nozzle. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations.

P27-112
Site – specific labeling of nucleotides in DNA strand for possible usage in electrochemical DNA-sensors

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Idea of electrochemical labeling of nucleotides and their incorporating to DNA strand firstly appeared in early 1980s. Until that time intrinsic elchem. properties of nucleobases were known, but

their applicability for analytical purposes was significantly limited. Introducing of labels has improved sensitivity and especially enabled to determine specific sequences by this way. And moreover, signals of these labels appear ussually in less extreme potencial than those from bases. Many such compounds have been succesfully tested [1–3]. Whereas many widely used methods requires expensive fluorescent labeling, cheapness and simplicity of elchem. devices can make them preferable in many types of analyses. If efficient method for such labeling and detection developed, it could allow construction of different types of probes and DNA sensors used in mol. biology analyses or for diagnostic purposes for example.

We incorporated single nitrophenyl group to one specific site in DNA strand and also combination of different labels to one DNA strand. We synthesized such modified DNA-strands with sufficient efficiency and proved, that they are detectable by adsorptive stripping voltammetry in 4 μM, resp. One micromolar input concentration of primer on Hg-electrode. There can be shown, that ratio of signals of individual tags corresponds well with number of such modified nucleotides in strand.

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P27-113
Neurotoxic response to silver nanoparticles in rats after repeated oral exposure

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There is an increasing interest in the health consequences of using materials which consist of silver nanoparticles. Despite of the fact that human exposure to nanosilver (AgNPs) is increasing, only a few studies is dedicated to researching of possible neurotoxic effect of AgNPs. Most of the previous studies on nanosilver toxicity have used *in vitro* models.

The aim of the present study was to determine whether this small- sized commercially available nanosilver induces neurotoxic effects in male, adult rats. Rats were exposed orally to 10 nm nanosilver in size for 14 days. Silver concentration in plasma was determined by mass spectrometry at 3, 5 and 8 hour after single administration. Using transmission electron microscopy (TEM), nanosized granules were detected in brain and comparatively in liver of exposed rats. In both tissues markers of oxidative stress were measured. Lipid peroxidation in brain and liver homogenates increased whereas the level of reduced gluthatione (GSH) decreased suggesting the involvement of oxidative stress in nanosilver- induced neurotoxicity.

P27-114
Homing of bone marrow and purified transplanted stem cells in marrow ablated mice

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The whole body irradiation of mice by lethal or sublethal doses represents a model for elimination of endogenous hematopoiesis and demonstration of efficacy of transplantation with hematopoietic cells. In our experiments we transplanted either unselected bone marrow cells or positively selected bone marrow cells $lin^-/CD117^+$ from GFP^+ donor. We performed a histochemical analysis of GFP^+ cell engraftment in the recipient's tissues. Tissue analyses were performed on day 30 and day 70 after exposure to 9 Gy irradiation and transplantation of GFP^+ bone marrow cells or $lin^-/CD117^+$ cells.

After 30 days, the transplanted cells were frequently observed in the vicinity to the endosteum of bone trabeculi in the bone marrow. Large numbers of grafted cells entered the thymic parenchyma. Lot of GFP^+ cells engrafted the lamina propriaof small intestines and splenic parenchyma.

After 70 days, numerous transplanted cells occupied the core of intestinal villi and splenic nodules in the splenic white pulp. In the liver, most GFP^+ cells differentiated into hepatic Kuppfer cells.

We also detected GFP gene expression in the recipient's tissues using qRT-PCR. After bone marrow transplantation, we detected increased levels of GFP in the spleen and in the thymus on day 30. On the other hand, transplantation of $lin^-/CD117^+$ cells resulted in the largest accumulation of engrafted cells in the spleen on day 30 followed by a transient decrease; next peaks were detectable on day 70 in the spleen and thymus.

Transplantation of lethally irradiated mice with bone marrow-derived cells supports hematopoiesis, but also leads to colonization of diverse organs with grafted cells.

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P27-115
Drug selected human lung cancer stem cells in non-small cell lung cancer

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Background: Tumor regeneration after chemotherapy is thought to be because of subpopulations of tumor cells termed cancer stem cells (CSCs), although it has been not confirmed yet. These cells are able to regenerate the tumor because of their high drug resistance and tumoroigenicity. We hypothesized that, CSCs could be enriched and isolated from tumor cell population following drug tretament.

Objective: The aim of our study was to isolate and characterize drug-selected human lung cancer stem cells.

Results: In primary screen compounds from Vichem's NCL, (library of kinase inhibitors) were screened for H358, A549, HCC827 Non-small Cell Lung Cancer (NSCLC) cell lines with *CellTiter-Glo* Luminescent Cell Viability Assay. The ratio of survived cells and the untreated samples (positive control) was examined. We further investigated the effective compounds which showed more than 75% inhibition in 10 μM and determined their IC50 and IC95 values for NSCLC cell lines. Compounds which had IC50 value lower than 2 μM were chosen for additional experiments. These hit compounds were screened for normal lung cell line (NL20) in order to exclude toxic molecules. Clonogenic assay was performed due to elucidate the colony forming ability and to describe the proliferative potential of drug selected CSCs. The apoptotic effect, and permeability of hit compounds were also determined. The migration and invasion potential, and the expression of cell-surface markers of the drug-selected cells were typified/described.

Conclusions: Promising lead molecules have been identified in order to achieve human lung cancer stem cells and these set of cells have been analysed on the basis of known CSC properties. In the future study we plan to analyse the cytokine network of these cells and claryfie whether the lead compounds are the target of ABCG2 transporter or not.

P27-116
NCU-G1 knock-out mice: a potential new model for liver fibrosis

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Liver fibrosis is a response to continued insults to the liver. Although different etiologies can lead to liver fibrosis, the progression and manifestations are very similar, and well characterized (1). Several animal models are widely used to study this disease, including classical methods that induce inflammatory responses in the liver through hepatotoxic agents, viral infections, and by bile duct ligation. In recent years, genetically modified animals, which are more susceptible to liver fibrosis, have been reported (2).

NCU-G1 is a novel protein which is well conserved throughout evolution. We initially described it as a potential regulator of transcription, while an other report identified it as a lysosomal membrane protein (3, 4).

To study the biological role of this potential dual-function protein, we created an *NCU-G1*$^{-/-}$ mouse model. *NCU-G1*$^{-/-}$ mice are lively, fertile, grow well, and have a similar life expectancy as their wild type siblings. However, liver damage and splenomegaly are detectable from an early age. The presence of α-sma positive cells, elevated hydroxyproline content, and the increased expression of the fibrosis inducer, TGF-β, in liver indicates fibrosis. Expression of several other fibrogenic genes are also elevated in *NCU-G1*$^{-/-}$ liver.

Liver damage is often induced or provoked in animal models for liver fibrosis. Here, we present a *NCU-G1*$^{-/-}$ mouse model, which spontaneously develops liver fibrosis at a young age. They are able to live with this condition for a normal life span, and could become a potential new model for studying this disease.

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P27-117
Occurrence of aflatoxins in layer feed and corn samples in Konya Province, Turkey

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Aflatoxins (B1 + B2 + G1 + G2) are a group of heterocyclic metabolites produced by fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxins (AF) frequently contaminate food and feed crops and have been a major concern as human hepatocarcinogens and as substances with potential deleterious effects on livestock health and productivity. In the Konya province of Turkey, layers (approximately 5 million hens) are commonly grown. Therefore, in the present study, the frequency and range of AF contamination in layer feed and corn samples is reported. The natural occurrence of aflatoxin was investigated in layer feed and corn samples brought to Konya Veterinary Control and Research Institute Laboratory. Seventy-eight samples (52 feeds, 26 corn samples) were analysed for total aflatoxin (B1 + B2 + G1 + G2) by an ELISA screening method. Aflatoxin contamination was determined in 37 feed samples (71.1%) and 15 corn samples (57.7%), with a range of 1.5–133 μg/kg. However, a majority of the aflatoxin contamination was < 5 μg/kg (50% within the positive samples). Two feed samples and two corn samples exceeded the maximum tolerated levels in feed (20 μg/kg) and feedstuffs (50 μg/kg) for total aflatoxin. Since AF are potent hepatocarcinogens for human and animals and cause significant economic losses, greater attention should be paid to the occurrence in feeds and feedstuffs.

P27-118
Serological and virological investigation of bovine viral diarrhea virus (BVDV) infection in cattle with abortion problem

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In this study, the presence of Bovine Viral Diarrhea Virus (BVDV) infection were examined in a cattle herd with abortion problem in Konya. Two hundred and twenty-eight blood serum and 228 leucocytes taken from cattle selected according to criteria for infertile and abortion problems were examined for antigens and antibodies to BVDV by Enzyme Linked Immunosorbent Assay (ELISA).

By ELISA, 41 (17.9%) sera were found seropositive and four (1.7%) leucocytes were BVDV antigen positive. Of these four BVDV antigen positive cattle, a number of two (0.8%) were detected seropositive while two (0.8%) were seronegative.

The animals being antigen positive and antibody negative were sampled second time after two weeks. The same results were detected for two seronegative cattle. The animals detecting persistant infection status were sent to slaugther.

It could be considered that both antigen and antibody positive animals might be sampled in acute phase of disease while antibody negative and antigen positive animals may be infected by *in utero* way during dam pregnancy.

Thirty-nine antibody positive and antigen negative animals could be infected by BVDV in any time of their lifespan. It is recommended that the animals should be checked in terms of BVDV for being negative both antigen and antibody before accepting them to the herds.

P27-119
Towards the understanding of the role of water in Biology: some answers from studies on organic water pores

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The presence of water molecules is recognized to be decisive in many biological phenomena such as recognition processes, transport across the membrane or aggregation, among others. However, the interplay between water molecules and biomolecules is not well understood. To a great extent, it is this interplay that ultimately enables life [1].

In the crowded intracellular medium, water molecules are often confined. In such situations, they exhibit different properties as compared to bulk water molecules. This kind of systems has been extensively investigated, very often by means of computational studies. Experimental models are needed to validate some of the obtained conclusions [2]. In our group, a family of organic hydroxyl-acids that self-organize giving rise to water pores of different diameters have been designed and synthesized. These pores provide appropriate experimental systems for the study of confined water [3].

We present recent results showing two different kinds of water molecules in our organic pores monitored by Far Infrared Spectroscopy. Depending on the pore diameter, they exhibit different dynamic behavior upon changes in temperature. Our findings are in agreement with recent studies on natural systems, where the release of water induces aggregation of oligomers related to the formation of amyloids [5].

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P27-120
Using biomaterials to create dynamic and well-controlled changes in the extracellular ionic environment

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Development of biomaterials that can successfully substitute damaged tissue either temporarily (i.e. the tissue engineering approach) or during prolonged time periods (i.e. permanent implants) requires close collaboration between physicians, cellular biologists, material scientists, and engineers. Although such research is mainly directed towards the creation of functional end products for *in vivo* applications, here it is explored how certain biomaterials also may be used to explore how cells respond to complex, but well-controlled changes of the biochemical *in vitro* environment.

For that purpose, the ion reactivity of a calcium phosphate based biomaterial (calcium-deficient hydroxyapatite) was first characterized using commercially available ion-selective electrodes, and also with miniaturized electrodes specifically designed for improved spatial and temporal control of the material-induced ion-exchange with standard cell culture media. Data was obtained on how the biomaterial influenced the activity of ions such as calcium, phosphate, and pH in the culture medium, and it could moreover be modeled mathematically from standard sorption theories.

Thereafter, osteoblast-like cells were grown on membranes that were permeable to ions and proteins so that cells and biomaterial could share the same aqueous environment, but still be physically separated from each other. In such conditions, material-induced adsorption of calcium and phosphate had only minor influence on cellular proliferation and alkaline phosphatase activity. However, competition for calcium and phosphate between cells and the biomaterial delayed and reduced significantly the deposition of calcium in the extracellular matrix.

P27-121
Investigation of apoptotic and efflux-pump gene expressions in Imatinib resistant CML cell line

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Overview: Chronic myeloid leukemia (CML) is a clonal disorder of the pluripotent hemopoietic stem cell, in which a reciprocal translocation t(9;22)(q34;q11) forms a Philadelphia (Ph) chromosome and creates a novel fusion gene, bcr–abl. Imatinib mesylate is one of the most widely used drugs for CML. Bcr-Abl can activate multiple signal transduction pathways which can promote cell proliferation and resistance to apoptosis. In addition, CML initiating cells may be innately resistant to therapies due to the expression profile of some efflux proteins. It is now well known that this resistance is closely related to different ABC efflux transporters such as MDR1, MRPs, BCRP. In the present study we examined mRNA expression levels of genes involved in drug transport (MRP1, BCRP, MDR1, OCT1) and apoptosis (BIM, BAD, BAX, BCL-2, TP53) in K562r (imatinib resistant) and K562s (imatinib sensitive) cells.

Methods: RNA isolation was performed with Trizol. Five hundred nanogram of total RNA was used for cDNA synthesis. Hprt expression was used as an internal control. Real time PCR was performed with Sybr green (95°C for 10 sec, 65–68°C for 30 sec and 72°C for 1 sec).

Results: The results showed that OCT1, BCRP, MDR1 genes mRNA expression levels in K562r cells was significantly higher than K562s cells. However MRP expression levels unchanged. Despite of an increase in expression of antiapoptotic gene BCL-2, proapoptotic genes BAX and BAD show low expression in K562r in comparison with K562s cells.

Discussion: These results suggest that, among all genes examined, especially BCRP and BCL-2 respectively showed 8.2 and 15.9 fold high expression levels which might indicate their essential roles in imatinib resistance of K562 cells.

P27-122
The role of the γTuRC subunit GCP8 in cytoskeleton organization

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Microtubules (MTs), tubular polymers of © and ®-tubulin, are part of the eukaryotic cytoskeleton and are essential for a variety of cellular functions, such as the formation of the spindle apparatus during cell division, directional transport of proteins and vesicles, cytoplasmic organization, cell polarization and motility. MTs are nucleated by a large ring-shaped protein complex, called the ©-tubulin ring complex (©TuRC). By mass spectrometry of affinity-purified ©TuRCs we have recently identified the novel subunit MZT2/GCP8. Unlike other ©TuRC subunits GCP8 is not required for ©TuRC assembly but contributes to ©TuRC recruitment and microtubule nucleation at interphase centrosomes. Interestingly, GCP8 depletion by RNAi does not lead to any obvious mitotic spindle defects, suggesting that GCP8 specifically affects the organization of the interphase microtubule network. Further analysis of GCP8-depleted cells suggests that GCP8 might also have a role in cell adhesion and migration and we are currently investigating the molecular basis of these phenotypes.

P27-123
Relationchips between taxonomy and function of microorganisms in natural environments

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Biochemical properties associated to soil microorganisms are critical to the functioning of the whole ecosystem. Several authors have postulated along the last decades that there is no relationship between taxonomy and function of microorganisms in natural environments: following Baas-Becking and Beyerinck '…for microbial taxa, everything is everywhere – but the environment selects'. This hypothesis does appear intuitive given that microbes can rapidly adapt to new environments and there are many thousands of species of microorganisms in a given soil (which implies a high degree of functional redundancy). However, data from high throughput sequencing in different environments suggest the opposite. In the present work we explore the existence of relationship between taxonomy and function of microorganisms in Mediterranean ecosystems in South-eastern Spain. Soil samples were characterized using biochemical parameters and microbial community structure estimated by the 16S rRNA gene analyses. Samples were collected at different altitude and under different land use and vegetation. Some biochemical parameters (microbial biomass carbon, soil basal respiration, initial inorganic nitrogen and enzymatic activities), allow differentiating soils according to their vegetation/use. However, nitrogen mineralization differentiates soils according to their altitude. Similarly, PCR-TGGE analysis revealed a differential behaviour in the microbial subpopulations studied which correlated with the biochemical pattern. A preliminary data set of 16S rRNA amplicon libraries generated from soil samples showed the presence of bacteria belonging to Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria, Verrucomicrobia, Gemmatimonadetes and Bacteroidetes. Our results suggest a relationship between the structure of microbial communities and the biochemical properties of soils enabling forest and degraded or cultivated soils to be differentiated. This implies that the molecular methods used could be applied to search for new soil quality indicators.

P27-124
Elevated number of circulating CD4+CD25highFoxp3+ T lymphocytes – new possible pathomechanism of disturbed immunoregulation in Transient Hypogammaglobulinemia of Infancy (THI)

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Transient hypogammaglobulinemia of infancy (THI) is a heterogeneous disorder with still unknown underlying basis. THI diagnosis is initially made after exclusion of other causes of hypogammaglobulinemia, while a definitive diagnosis can be only made retrospectively in patients with normalized IgG levels, usually between 2nd and 4th year of life. In this study we determined the absolute numbers of Treg in a large group of THI (n = 68), CVID (n = 32), sIgAD (n = 40) patients and age-matched control subjects (n = 65). THI patients had elevated number of circulating T_{regs} in comparison to other groups, in which the numbers of T_{reg} remain at the constant level, independently of age. Additionally, within THI group differences in the level of T_{regs}, depending on patient age were observed. The younger children had higher absolute numbers of T_{regs} (mean age 1.47 ± 0.79 years, mean T_{regs} – 87 cells/μl) than older children (mean age 2.45 ± 1.06 years, mean T_{regs} – 14 cells/μl). Interestingly, elevated T_{regs} numbers observed in younger children were only temporal and decreased with age to the values observed in healthy children. Furthermore, follow-up studies performed on 14 THI children suggest that decreasing number of T_{regs} over time is associated with normalization of immunoglobulin level. Our findings may have clinical implications, since the differential diagnosis between THI and CVID remains difficult due to the overlapping clinical and immunological features. Thus, the circulating T_{reg} number determination may be helpful in predicting THI, but not CVID, development. These observations may also suggest the new possible pathomechanism of disturbed immunoregulation in THI.

P27-125
Effect of methylsulfonylmethane on iNOS gene methylation in macrophages

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Overview: DNA methylation is an important biochemical and epigenetic process which plays roles in normal organismal development and cellular differentiation. Methylation of DNA occurs by the addition of a methyl group to 5th position of cytosine or 6th position of adenine. Recently, gene methylation has been

linked with progression of human cancers and chronic inflammation. In the present study methylation status of DNA in LPS/IFN-γ induced RAW 264.7 macrophages and effect of methylsulfonylmethane (MSM) on the methylation status was examined.

Methods: Bisulfite modification of DNA isolated from unstimulated cells, LPS/IFN-γ stimulated cells and MSM treated stimulated cells was executed by CpGenome DNA modification kit. After modifiaction, methylation specific PCR was used to identify the methylation status of iNOS promotor region. CpGenome Universal Methylated/Unmethylated DNA was used as +/- control. Primers for iNOS gene was designed with PerlPrimer programme. PCR products were loaded and seperated with %2 agarose gel.

Results and discussion: MS-PCR results showed that the methylation profiles of CpG island cytosines in LPS/IFN-gamma treated and MSM plus LPS/IFN-gamma treated cells remained unchanged in all samples. A wider region of iNOS promoter will be examined by bisulfide sequencing for further studies.

P27-126
GSTP1 gene methylation analysis in prostate cancer

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Prostate cancer (PCa) is the most common malignancy and the second leading cause of cancer death among men in the United States. Like other human cancers, prostate cancer development and progression are driven by interplay of genetic and epigenetic changes. Many CpG islands have been identified to be hypermethylated in prostate tumors, resulting in promoter inactivation and gene silencing. The GSTP1 gene encodes a detoxification enzyme involved in cell protection from carcinogens. CpG island methylation of glutathione-S-transferase-Pi (GSTP1) has been detected in several cancer types, including breast and hepatocellular cancer and PCa. In this study we aimed to analyze the relationship between the promoter methylation and inactivation of GSTP1 gene in prostate cancer. The study was performed on 142 patients which were consisted of 37 malign and 105 benign subjects. Genomic DNAs were isolated from peripheral blood samples. Isolated DNAs were modified by bisulfide treatment-DNA modification method. In the bisulfide reaction, all unmethylated cytosines were deaminated and sulfonated, converting them to Uracils, while 5-methylcytosines remain unaltered. Methylation of DNAs of 142 patients were analysed by Methylation Spesific PCR (MSP). Finally, methylated and unmethylated allells were analysed by %2 agarose gel electrophoresis. As a result, we could not find any relationship between GSTP1 gene methylation and malignancy in DNAs isolated from blood samples of prostate/prostate cancer patients. According to our findings GSTP1 methylation analysis can not be used as a biomarker in peripheral blood cell based experiments.

P27-127
Domain configuration and phylogeny of fatty acid synthases and polyketide synthase-like proteins in protists

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Fatty acids are essential components of all organisms. They are biosynthesized by fatty acid synthases (FASs), which use five enzymatic activities in an iterative manner: ketoacyl synthase (KS), acyl transferase (AT), keto reductase (KR), dehydratase (DH) and enoyl reductase (ER). The growing carbon chain is bound to an acyl carrier protein (ACP). The configurations of these activities are different among organisms. Type II FAS systems (FAS-II) seem to be the evolutionary oldest. They have the activities located on distinct proteins and are typical for prokaryotes. Type I FAS systems (FAS-I), however, consist of long protein chains that comprise several of the enzymatic activities. They are characteristic for both animals and fungi but the domain configuration differs. Polyketide synthases (PKSs) represent a biochemically similar enzyme system, which produces structurally highly diverse secondary metabolites, while catalyzing exactly the same reactions as FASs. The distribution of these enzyme systems in bacteria, plants, fungi and animals shows a clear pattern, but the situation in protist organisms is much less understood. Using data base collections and our own sequencing data, we have analyzed the distribution and domain configurations of FAS types and PKS-like proteins in diverse groups of protists. Furthermore, we reconstructed their phylogenies based on the KS domains. Our results show that FAS and PKS-like proteins in protists went through a long common evolutionary history and can have unusual domain configurations not found in other organism groups. Distribution patterns seem to be more irregular than in bacteria and higher eukaryotes.

P27-128
Next generation sequencing in clinical practice

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Massive parallel sequencing allows one to sequence an individual human genome at a reasonable cost. This not only has enormous significance for scientific research and genetic diagnostics, it also presents society with a number of new challenges. From the scientific perspective, such information can facilitate the faster identification of new genetic conditions. From the clinical perspective, it is probable that total genome analysis will occupy a central place in medical practice of the future. In addition, our knowledge of the individual genome will allow us to make genetically determined choices in terms of lifestyle and procreation. But reading is not the same as understanding: the analysis of a genome is complex and the function and importance of a great many genetic variations remains a mystery. There is thus a considerable difference between raw and analyzed information. The clinical and familial context of a patient or individual is indispensible in the process of interpretation, and the results are often not unambiguous. The more extensive the test, the greater the chance that one will encounter information one was not looking for. 'Incidental findings' relate to genetic information with a level of clinical utility, outside the immediate medical context of the condition being explored (e.g. predisposition for hereditary cancer syndromes or neurodegenerative conditions). The best solution is that the doctor raises the question of potential incidental findings prior to conducting the test.

A practical solution for dealing with the abundance of genetic information might be the implementation of filters. Filters are employed to facilitate the classification of different variants. In the present context, they could be used to separately classify sought after and non-sought after findings. They may also be useful in masking the thousands of genetic variants detected in the genome of a given patient, the significance of which is not yet known.

At the University of Leuven, we have established a number of recommendations on total genome analysis. They will be presented at this symposium.