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Abstracts submitted to the 41st FEBS Congress, which was planned for Kuşadası, Turkey from 3rd to 8th September 2016, and accepted by the Congress Organizing Committee are published in this Special Issue of *The FEBS Journal*. Unfortunately, the Congress was cancelled by FEBS after the excellent scientific programme was compromised by an insufficient number of confirmed speakers, and so the authors of these abstracts were not able to present their work at the event\*. Late-breaking abstracts and abstracts withdrawn after Congress cancellation are not included in this issue.

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Abstracts submitted to the Congress are **not peer-reviewed**. In addition, abstracts are published as submitted and are **not copyedited** prior to publication.

We are unable to make **corrections of any kind** to the abstracts once they are published.

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\*\* The Abstract number begins with either the letters S or ML and can be found atop each abstract's title in the PDF file.

# SYMPOSIA

**Sunday 4 September**  
**9:00–11:00, Hall A**

## **DNA replication and recombination: Novel aspects**

**S-01.01.1-001**

### **Key mechanism in the loading and activation of the replicative helicase MCM2-7**

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Initiation of eukaryotic DNA replication is divided into two phases, the loading of the replicative helicase MCM2-7 onto origin DNA and its activation. During helicase loading, also termed pre-RC formation, the six-subunit origin recognition complex (ORC), cell-division-cycle 6 (Cdc6) and Cdc10 protein-dependent transcript 1 (Cdt1) cooperate to load the helicase onto dsDNA as a MCM2-7 double-hexamers. This multi-step process can be arrested at an early stage using a non-hydrolysable ATP analogue. In the absence of ATP-hydrolysis an ORC/Cdc6/Cdt1/MCM2-7 complex (OCCM) is formed, with DNA passing through the hexameric MCM2-7 ring. DNA insertion depends on the opening of the MCM2-7 ring at the Mcm2/Mcm5 interface. Here we have studied the *Saccharomyces cerevisiae* OCCM complex using biochemical and electron microscopy approaches. A 3.9Å electron microscopy structure and a cross-linking mass-spectrometry analysis revealed the organisation of this 14-subunit 1.1 MDa complex on DNA, providing critical insights into eukaryotic replicative helicase loading. We discovered that in the OCCM, Mcm2-7 winged helix domains (WHD) form flexible interactions that tightly engage with ORC/Cdc6. Cdt1 presented itself in an extended three-domain configuration that embraces Mcm2, Mcm4, and Mcm6, nearly half of the hexamer. The Cdt1 C-terminal domain struts the Mcm6 WHD, which in turn binds Orc4 WHD. Importantly, the double-stranded DNA passes straight through the ORC-Cdc6 ring, but then enters the Mcm2-7 ring in a ~25° bent conformation. The DNA interaction is mediated by a budding yeast specific  $\alpha$ -helix in Orc4 and two positively charged loops in Orc2 and Cdc6. In this intermediate, the Mcm2-7 C-tier AAA+ ring is topologically closed by a Mcm5 loop that embraces Mcm2, but the N-tier ring is open at Mcm2-Mcm5 interface. In summary, this structure sheds light in the loading mechanism of the first Cdt1-bound Mcm2-7 hexamer by ORC-Cdc6.

**S-01.01.1-003**

### **Overcoming DNA topological stress during DNA replication to prevent genome instability**

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The structure of the DNA double helix dictates that the two strands of nucleic acid are intertwined every 10.4 basepairs.

Faithful genome duplication and inheritance requires the complete resolution of all intertwinings, across every parental chromosome in a cell. This is achieved by topoisomerase action ahead of the replication fork or by fork rotation and subsequent resolution of the DNA catenation formed. Although fork rotation predominates at replication termination, *in vitro* studies have suggested that it also occurs frequently during elongation. However the factors that regulate fork rotation and how it might influence other replication associated processes are unknown. Here we examine the contexts and factors that regulate fork rotation in the yeast *Saccharomyces cerevisiae*. We find that fork rotation is restricted during elongation by the evolutionarily conserved Top1/Csm3 proteins. These proteins prevent fork rotation from braiding the sister chromatids and causing DNA damage in the wake of the fork. However, we do find that some fork rotation takes place during elongation at stable protein-DNA complexes when Top1/Csm3 is present, at the expense of inflicting DNA damage at these known fragile sites. We conclude that although fork rotation is required in distinct contexts to facilitate replication, it is an intrinsically de-stabilising process that is kept restricted by replisome structure and associated factors.

**ML-01.01.1-001**

### **PRDM9 is not the only major regulator factor for the human crossover hotspot DA**

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Meiotic recombination plays a key role in reshuffling haplotypes in human populations. However, our understanding of recombination dynamics is largely limited to descriptions of variation in populations and families. PRDM9 has a critical role in specifying meiotic recombination hotspots location in humans and mice via recognition of hotspot sequence motifs. To examine the effects of both 13-bp motif (cis-regulator) and trans-regulator PRDM9 on crossover frequencies and distribution, we studied Hotspot DA identified from HapMap data. This hotspot had the motif at its centre, and a SNP that disrupts the motif. LDU analysis confirmed the location of the putative hotspot to a 1–2-kb interval. A 15-kb target interval around Hotspot DA hotspot sequence motif was assayed for recombination activity in six men. The crossover frequency showed Hotspot DA to be a regular hotspot with an average crossover rate ( $\sim 8 \times 10^{-4}$ ) among hotspot assayed on autosomes. Comparing the rates and distributions of sperm crossover events between donors heterozygous for the disrupting SNP showed that there was a huge asymmetry between the two alleles, with the derived, motif-disrupting allele completely suppressing hotspot activity. Intensive biased gene conversion, both into crossovers and non-crossovers, has been found at Hotspot DA. Biased gene conversion that influences crossover and non-crossover activated hotspot activity correlates with PRDM9 allele A. In Hotspot DA, the lifetime of the hotspot mostly depends on the cis-regulatory disrupting SNP DA7.5, and on the trans-regulatory factor PRDM9. To conclude, Hotspot DA, is the only

evidence for human crossover hotspot regulation by a very strong cis-regulatory disrupting SNP.

### ML-01.01.1-002

#### Human mitochondrial genome can replicate in the *Yarrowia lipolytica* yeast

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The absence of homology recombination in mitochondria (MT) is well known. Therefore, the resident MT genome is inaccessible for genetic engineering modifications. Such opportunity is of great practical importance for treating MT pathology in human. Our study supplies proofs of possibility of replicating the intact h-MT genome in the *Y. lipolytica* yeast (*Yl*).

The experiments included six stages.

RecA gene from *subtilis* was expressed in *Yl* W29 using SOD2 promoter and terminator. The SOD2 elements provided signals responsible for RecA translocation to *Yl*-MT.

MT-adapted hygromycin (Hyg) resistance marker was designed. It bears ND1 translation initiation signal and no promoter (the gene was intended for expression within *Yl*-MT genome operon 1).

Leu36H and Lys39H constructs for replacement of tRNA-Leu and tRNA-Lys genes in *Yl*-MT genome were obtained. Both constructs were transformed to *Yl* W29 (MT-RecA+). The transformants were chosen using Hyg. The integration of both constructs to *Yl*-MT genome by homology was confirmed by PCR. The integrants were able to grow in complete medium only but not in minimal medium with succinate as carbon source.

Leu36H and Lys39H bearing clones were used for transformation of the total human genome (including MT). The transformants were selected using minimal succinate medium.

h-MT genome copy number was assessed in *Yl* W29(Leu36H) and *Yl* (Lys39H) derived transformants using qPCR. Roughly equal copy number of the *Yl*-MT and h-MT genomes in the transformants was revealed. Loss of a single h-MT marker was found in 2 clones of 8 and in 1 of 7 clones.

The ability of the h-MT genome to replicate in the yeast was shown. This is confirmed by phenotypical complementation of tRNA-Leu and tRNA-Lys defects of *Yl*-MT genome by h-MT homologues. The engineered system is applicable for *ex vivo* correction of mutations associated with MELAS and MERFF. Acknowledgments: Supported by the Russian Federation Ministry of Science and Education (Grant No RFME-FI60414X0112);

**Sunday 4 September**

**9:00–11:00, Hall B**

### Nuclear architecture

#### S-02.01.1-003

##### 4D genome dynamics

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Three-dimensional chromatin organization is tissue-specific and plays multiple roles in control of genome functions. However,

individual cells show a high but finite level of variability in chromosome conformation and genome organization, which limits the interpretive power of cell population based experiments. To create a better understanding of how genome structure relates to function we have developed single cell Hi-C. Our original Hi-C experiments were characterized by sparse genomic coverage and low cell throughput. We studied the single X chromosome of male Th1 cells from an inbred strain of mice. We showed that the genome conformation of all cells showed evidence of topological domain structures (TADs), and that chromosome structure varied significantly from cell to cell at the level of long-range inter-TAD interactions. We showed that active domains tended to locate to the surface of chromosome territory structures and preferentially contacted other active TADs via robust inter-chromosomal contacts. Lamin associated domains (LADs) also tended to locate to the surface of chromosome territories but were devoid of inter-chromosomal contacts, and were often found on one side of the chromosome territory models consistent with chromosome positioning adjacent to the nuclear lamina. Here we present the next generation of single cell Hi-C experiments on haploid and diploid F1 hybrid mouse ES cells. We have dramatically improved both genomic coverage and cell throughput and will present and discuss ongoing analyses and results from thousands of single cells, which illustrate the dynamics of genome conformations.

#### S-02.01.1-002

##### Deconstruction approaches to study genome architecture and function

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One of the central challenges in genome biology is to understand how the linear organization of the genome (e.g., the order and spacing of genes and the relative positions of regulatory elements) contributes to gene regulation and 3D chromosome organization. To tackle this challenge, we need efficient means to systematically perturb the linear organization and then use genome-wide readouts to study the functional and structural consequences. We are developing two complementary strategies to achieve high-throughput perturbation of the linear genome. We combine these methods with our DamID approach to study interactions of the genome with the nuclear lamina.

First, we have developed a multiplexing method to assay the regulatory activity of small (0.2–2 kb) genomic fragments when taken out of their genomic context. We achieve an extremely high throughput (>100 million promoter activity assays). The resulting dataset can be used to separate regulatory activity encoded in local sequences from long-range and chromatin-mediated regulation. We are using this information to identify genes that are subject to chromatin effects in lamina-associated domains in human cells.

Second, we are developing an approach to "scramble" the genome of mouse ES cells, by inducing a large number of random inversions and deletions. For this we use randomly integrated nested transposable elements that carry LoxP recombination sites. Transient expression of Cre then causes a large number of rearrangements. We have designed a cost-effective sequencing approach to map these arrangements. Our aim is to produce a large series of clonal ES cell lines that each carry dozens of precisely mapped rearrangements. This cell line collection will be used to study the cis-determinants of spatial genome organization, for example by mapping changes in genome – nuclear lamina context.

**S-02.01.1-001****Chromatin dynamics during transcription initiation at single gene loci**

K. Bystricky

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The spatial organization of chromatin in the nucleus is non-random and chromatin dynamics participate in regulating nuclear processes from gene expression to DNA repair. Transcriptional activity has been correlated with relocalisation of gene loci within the cell nucleus. However, we do not know whether changes in transcription *per se* alter motion of the underlying chromatin fiber.

We use estrogen inducible loci in human mammary tumour cells as a model system in which chromatin remodelling via looping allows priming of the gene environment for transcription activation. We mapped chromatin folding over several hundred kb around estrogen responsive genes using 3D DNA and RNA FISH and confronted these measurements with 5C data to establish models of domain organization which are cell type specific. Our results indicate that rapid estradiol induction of gene expression occurs in the context of pre-existing chromosomal architectures that become stabilized in response to estradiol signalling. Using the non-invasive ANCHOR™ method to label DNA for imaging chromatin in living human cells, we follow chromatin dynamics of a specific gene locus during the first 30 min of transcription activation. Simultaneous observation of mobility and transcription of a single, hormone-responsive gene, CyclinD1 showed high cell-to-cell variability. Addition of estradiol caused a rapid decline in chromatin motion, prior to detection of new mRNA and regardless of pre-induction mobility. Inhibition of transcription elongation did not fully restore chromatin motion, indicating that as soon as RNA polymerase II initiates transcription the CyclinD1 gene domain undergoes major conformational changes that reduce its mobility. Our observation that transcription initiation locally reduces chromatin dynamics within minutes is compatible with the idea that existing chromatin conformation reorganizes to facilitate enhancer promoter contacts and chromatin de- and reassembly.

**Sunday 4 September****9:00–11:00, Hall C****Systems biology****S-03.01.1-002****Evolutionary tradeoffs and the geometry of gene expression space**

U. Alon

*The Weizmann Institute of Science, Rehovot, Israel*

Organisms, tissues and cells often need to perform multiple tasks. But usually no phenotype can be optimal at all tasks at once. This leads to a fundamental tradeoff. We study this using the concept of Pareto optimality from engineering and economics. Tradeoffs lead to an unexpected simplicity in the range of optimal phenotypes—they fall on low dimensional shapes in trait space such as lines, triangles and tetrahedrons. At the vertices of these polygons are phenotypes that specialize at a single task. This one can infer the evolutionary tasks directly from the data. This is a new approach to understand big datasets with many numbers and many samples, especially data that can not be easily clustered— with online software freely available. We demonstrate this using data from animal and fossil morphology, gene expression data from bacteria, single human cells and tumors, and other biological systems.

**S-03.01.1-003****Respect the noise: exquisite interaction of cellular noise and dynamics lead to novel biological function**

M. H. Khammash

*Kenes, Istanbul, Turkey*

Using homeostatic regulation and oscillatory entrainment as examples, I demonstrate how novel and beneficial functional features can emerge from exquisite interactions between intracellular noise and network dynamics. While it is well appreciated that negative feedback can be used to achieve homeostasis when networks behave deterministically, the effect of noise on their regulatory function is not understood. Combining ideas from probability and control theory, we have developed a theoretical framework for biological regulation that explicitly takes into account intracellular noise. Using this framework, I will introduce a new regulatory motif that exploits stochastic noise, using it to achieve precise regulation and perfect adaptation in scenarios where similar deterministic regulation fails. Next I propose a novel role of intracellular noise in the entrainment of decoupled biological oscillators. Thus in both regulation and oscillatory entrainment, beneficial dynamic features exist not only in spite of the noise, but rather because of it.

**S-03.01.1-001****Dissecting the complexity of cancer signalling**

N. Bluthgen

*Charite, Berlin, Germany*

Many tumours are driven by alterations in the EGFR signalling pathways, and targeted therapies that block components in these pathways are increasingly used as first or second treatment line. Yet, often tumour cells display intrinsic resistance to these therapies that are difficult to understand. Here we discuss how different topological features of the signalling networks such as feedbacks and feed-forward loops contribute to resistance. Using perturbation data of signalling combined with mathematical modelling, we show how combinatorial treatment can help to overcome resistance, and potentially also avoid development of resistance.

**ML-03.01.1-001****An integrative approach to analyze dynamic transcriptional response of yeast cells to DNA damage**M. E. Karabekmez<sup>1,2</sup><sup>1</sup>*PHI Tech Bioinformatics, Kocaeli, Turkey*, <sup>2</sup>*Department of Chemical Engineering, Bogazici University, Istanbul, Turkey*

DNA damage triggers transcriptional response of diverse biological processes other than DNA repair. *Saccharomyces cerevisiae* is a well defined and easy to manipulate model organism. Until the last two decades the most of the information on DNA damage was coming from yeast. Most of the related studies use static approaches while some others measured dynamic transcriptional response of yeast cells as well but these works mostly focus on other biological problems instead of using systems biology approaches at genomic level to get a complete picture of the DNA damage response. In this study, time series microarray datasets collected after a genotoxic stress in *Saccharomyces cerevisiae* were selected from the literature. Differentially expressed genes were identified by a novel approach and resulting genes were analyzed by a novel pipeline constructed by using well-

established clustering methods, integrative tools with modifications. Vacuolar and proteasomal ubiquitin-dependent protein catabolic processes, cellular response to heat and DNA repair processes were found to be induced after exposure to DNA damage. Interestingly base-pair excision repair mechanism could not be identified as responsive to DNA damaging agents. Ribosome biogenesis, cell cycle, DNA packaging, nucleosome organization chromatin assembly biological processes and mitosis related genes were identified to be down regulated upon exposure to DNA damaging reagents. Some of the genes related to response to drug process were found to be induced in response to DNA damage whereas some specific genes involving in drug trans-membrane transport were found to be irresponsive to UV. Arginine biosynthesis related genes were also identified to be up-regulated whose relation to DNA damage response is needed to be investigated further. It can be concluded that different sources and doses of DNA damage might induce or inhibit distinct biological processes.

### ML-03.01.1-002

#### Genomic meta-analysis and discovery of new biomarkers in colorectal cancer

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Colorectal cancer (CRC) is one of the most prevalent tumors worldwide with the third highest mortality rate in developed countries. Early diagnosis is crucial to the treatment and prevention of CRC, yet the absence of clear symptoms at its onset makes early diagnosis almost impossible. To date, the most plausible diagnosis tool for screening this type of malignancy is the use of biomarkers, however there is need to identify new biomarkers due to inadequacies of the current ones. Gene expression profiling studies are crucial in the identification of probable CRC biomarkers hence a meta-analysis was performed comparing 98 samples of our discovery set against 1174 samples of a validation set from 10 studies of the GEO and Array express databases (Affymetrix platform) so as to validate our findings and derive more robust conclusions. Corresponding subgroups of both sets were analyzed separately after sample normalization using RMA methodology embedded in the Partek<sup>®</sup> Genomics Suite<sup>™</sup> 6.6 software and differentially expressed genes were determined at the  $P \leq 0.001$  cut off with false discovery rates and enrichment score  $\geq 1.3$  was applied during clustering.

The results indicated a 60% validation of the ANOVA tumor vs normal gene-list of the discovery set. Meanwhile, DAVID bioinformatics tool analyses related significant enriched clusters to pathways such as Pathways in cancer, Focal adhesion and Aldosterone regulated sodium reabsorption. A 9-gene signature was highlighted as possessing diagnostic potential in CRC patients. Grade gene-list overlaps validated 66% of grade II vs normal and 68% of grade III vs normal of our cohort. Traces of pre-invasive genes such as CCND1, MYC, and CDK1 were identified in grade II while grade III had a complex composition of genes including PPAR $\alpha$ , CDKN2A and GPX1.

Therefore this study may be useful in providing more insight into scantily studied genes believed to have a role in CRC grading.

## Sunday 4 September

9:00–11:00, Hall D

### Host–pathogen interactions

#### S-04.01.1-001

#### A central contribution of lipid mediated signaling in *Toxoplasma gondii* egress from host cells

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The phylum Apicomplexa is composed of a large group of obligate intracellular parasites that cause severe veterinary and human diseases including *Plasmodium* spp., the etiologic agent of malaria and *Toxoplasma gondii* responsible for toxoplasmosis. Gliding motility, a substrate-dependent form of locomotion, powered by an actomyosin system assists invasion and egress from the infected cells, two key steps in the lytic cycle of the Apicomplexa. Exit from the host cells is recognized as a complex, well-orchestrated and temporally controlled process. Underpinning this process is the release of apical secretory organelle termed micronemes. Activation of the cGMP dependent kinase and an increase in intracellular calcium levels, likely resulting from parasite PI-PLC activation at the plasma membrane, activate calcium-dependent protein kinases (CDPKs) that subsequently phosphorylate specific substrates, ultimately leading to microneme exocytosis. Downstream of PI-PLC activation, diacylglycerol kinase 1 (DGK1) produces phosphatidic acid (PA) from diacylglycerol (DAG) and contributes via membrane protein recruitment and possibly via membrane curvature to microneme exocytosis. In this context, the apical pleckstrin homology domain containing protein, APH is acylated at the surface of the micronemes and acts as an essential PA sensor leading to organelle fusion at the plasma membrane. Intriguingly, *T. gondii* possesses a second diacylglycerol kinase (DGK2), which secreted into the parasitophorous vacuole where it plays an instrumental role in breaking the parasitophorous vacuole membrane during parasite egress via a yet unknown mechanism.

#### S-04.01.1-003

#### How cells defend their cytosol against bacterial invasion

F. Randow

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Intracellular pathogens inhabit specific cellular niches determined by the degree of compartment-specific immune surveillance and the pathogen's need for host cell activities and nutrients. Most intracellular bacteria dwell in vacuoles while only few have conquered the cytosol, a perhaps counterintuitive situation considering the abundant energy sources available in the cytosol for bacterial growth. Potent cytosolic defense mechanisms must therefore exist. I will discuss the role of autophagy in defending the cytosol from bacterial invasion, in particular how 'eat-me' signals including galectins and ubiquitin become associated with cytosol-invading bacteria, how cargo-selecting autophagy receptors target cytosolic bacteria for destruction, and how professional cytosol-dwelling bacteria escape from autophagy.

**S-04.01.1-002****Hijacking of cellular pathways by the Nipah virus matrix protein: insights into paramyxovirus biology from the deadliest virus you've never heard of**

R. Watkinson<sup>1</sup>, Y. Wang<sup>2</sup>, M. Pentecost<sup>1</sup>, A. Park<sup>1</sup>, A. Vashisht<sup>2</sup>, T. Yun<sup>3</sup>, A. Freiberg<sup>3</sup>, J. Wohlschlegel<sup>2</sup>, **B. Lee<sup>1</sup>**  
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Nipah virus is a highly lethal emergent Paramyxovirus responsible for repeated human outbreaks of fatal encephalitis in South East Asia. The WHO has designated Nipah virus as one of the top 5–10 emerging pathogens likely to cause severe outbreaks in the near future. Initial zoonotic transmission can occur directly from the natural bat reservoir, or via an intermediate host such as domesticated swine, but subsequent human-to-human transmission is well documented. There are no approved vaccines or treatments for Nipah virus, thus improved understanding of the molecular cell biology of its life cycle is critical for identification of potential therapeutic targets. The Nipah virus matrix protein scaffolds budding of nascent virions at the plasma membrane, recruiting a plethora of cellular machinery to efficiently coordinate particle assembly. Intriguingly, matrix also hijacks the cellular nuclear-cytoplasmic trafficking and ubiquitination pathways to facilitate transient nuclear localization that is a prerequisite for subsequent budding. This is unexpected as paramyxoviruses are known to replicate entirely in the cytoplasm. While the biological significance of nuclear localization of the matrix protein of an otherwise cytoplasmically replicating virus remains enigmatic, the molecular details have begun to be characterized, and are conserved amongst matrix proteins from several divergent Paramyxovirus genera, including the parainfluenza viruses that cause significant disease burden in children. Matrix protein appropriation of cellular machinery will be discussed, both in terms of its early nuclear targeting, and later role in virion assembly. We will also share insights on the cell biology of matrix protein using data from the matrix interactomes of Nipah virus and other paramyxoviruses.

**ML-04.01.1-001****Elucidation of subcellular localization and function of *Puccinia striiformis* f. sp. *tritici* effectors**

**B. Dagvadorj<sup>1</sup>**, T. O. Bozkurt<sup>2</sup>, A. C. Ozketen<sup>1</sup>, A. Andac<sup>1</sup>, M. S. Akkaya<sup>1</sup>

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Determination of pathogen candidate effectors is the focus of the plant-pathogen interaction studies in plant molecular biology. Understanding the molecular details of pathogen secreted effectors would allow to generate novel strategies in the fight against plant diseases, especially for sustainable crop production. Recent, rapid advances in next generation sequencing of the genomes and transcriptomes of many disease-causing pathogens are facilitating the accumulation of information on the pathogen candidate effectors. One of the major crop diseases worldwide is yellow rust of wheat. The agent is biotrophic fungus *Puccinia striiformis* f. sp. *tritici* (PST) with capability of causing epidemics in all the continents. There are two layers of plant immunities including PAMP-triggered immunity (PTI) and Effector-triggered immunity

(ETI). In PTI, pathogen associated molecular pattern (PAMP) molecules activates plant innate immune system being recognized by surface proteins. However, to promote infection, effector molecules from the adapted-pathogens suppress this defense, named effector-triggered susceptibility (ETS). On the other hand, in disease resistant plant cultivars, plant resistance (R) gene products detects the effectors and activates ETI, which is much more stronger immunity than PTI.

In this study, we used *Nicotiana benthamiana* as a model organism for transient gene transfer by agro-infiltration of candidate effectors as tagged and/or GFP fused constructs, which were cloned using gateway technology, followed engineering with PCR of the genes had made synthesized.

The three candidate effectors were selected based on producing a small, cysteine rich and signal peptide containing proteins. Each localized differently; one was identified as targeting chloroplast, another was secreted into plant cytoplasm, and the last one was secreted to apoplast where it resulted in cell death upon over expression, suggesting it is involved in PAMP triggered immunity.

**ML-04.01.1-002****A novel Abl kinase-dependent cellular entry mechanism of *Pseudomonas aeruginosa* via a glycosphingolipid receptor**

**S. Zheng<sup>1,2</sup>**, T. Eierhoff<sup>1,2</sup>, W. Römer<sup>1,2</sup>

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*Pseudomonas aeruginosa* (*P. a.*) is a Gram-negative opportunistic human pathogen, which causes severe infections of the respiratory tract, urinary tract, skin and eyes. Internalization of *P. a.* by host cells significantly contributes to its pathogenicity. The entry mechanism(s) and the host cell factors involved in this process are incompletely understood. The galactophilic lectin LecA, one of the virulence factors produced by *P. a.*, acts as an invasion factor and interacts with globotriaosylceramide (Gb3), a host cell glycosphingolipid (GSL), triggering membrane engulfment of *P. a.* at the initial stage of entry into human lung epithelial cells. Previous studies have shown that Abelson tyrosine kinase (Abl), a non-receptor tyrosine kinase, promotes cellular uptake of *P. a.* However, the bacterial factors as well as the host cell receptors, which activate Abl signalling during *P. a.* invasion, remain elusive.

We employed human lung epithelial cells H1299, Chinese hamster ovary (CHO) cells and the *P. a.* wild type strain PAO1 to study the invasion process of *P. a.* into host cells by using microbiological, biochemical and cell biological approaches.

Our results corroborated that Abl kinase activity is required and induced for PAO1 efficient entry into H1299 cells. LecA strongly triggers Abl kinase activity even at nanomolar concentrations in H1299 cells. Ectopic expression of Gb3 in CHO cells sensitized these cells for LecA-induced Abl activation. Also, Src family kinases have been linked to LecA-Gb3 mediated Abl activation.

We identified a glycolipid receptor, Gb3, for Abl kinase activation, and demonstrated a yet undescribed role for LecA during the cellular invasion process of *P. a.* LecA-Gb3 interactions not only trigger initial, bacterial membrane engulfment but also induce Abl-dependent signalling to promote efficient bacterial entry into host cells. Hence, this lectin-GSL complex may represent a potential target for drug development.

Sunday 4 September

9:00–11:00, Hall E

**DNA repair and cancer****S-05.01.1-003****Repair mechanisms for endogenous DNA damage****D. Wilson***National Institute on Aging, NIH, Baltimore, Maryland, United States of America*

Our genetic material is susceptible both to spontaneous hydrolytic decay and to reactions with natural intracellular chemical species, namely oxygen radicals or reactive aldehydes. The spectrum of so-called endogenous DNA damage comprises simple and bulky base modifications, abasic sites, non-conventional single-strand breaks, and complex lesions, such as interstrand crosslinks (ICL), to name a few. To counteract the lethal and mutagenic effects of DNA damage, cells have developed an array of repair mechanisms, including base excision repair (BER) and transcription-coupled processes. I will touch upon the potential role of a central participant in BER, i.e. apurinic/apyrimidinic endonuclease 1 (APE1), in disease risk, and the emerging contribution of the proteins defective in the premature aging disorder Cockayne syndrome to ICL resolution.

**S-05.01.1-001****Base excision repair and cancer****J. Sweasy***Yale University, New Haven, United States of America*

Endogenous DNA damage occurs at rates exceeding 20 000 DNA lesions per cell per day. The base excision repair (BER) machinery has evolved to repair endogenous DNA damage and is necessary to maintain genomic stability and prevent cancer. There are hundreds of single nucleotide polymorphisms (SNPs) and tumor-associated somatic mutations predicted to result in a phenotype in the genes that encode BER proteins in humans. Our laboratory has been characterizing many of these SNPs and somatic mutations. Our methods include *in vitro* biochemical studies of glycosylase and polymerase activity, structural analysis, and cell biological approaches using predominantly MCF10a immortal human breast epithelial cells, but also various other cell lines. We have found that variant DNA glycosylases, DNA polymerase beta enzymes, and XRCC1 encoded by these genetic variants result in proteins that are unable to catalyze BER efficiently. Expression of these protein variants in cells results in genomic instability that leads to cellular transformation. Genomic instability usually stems from the accumulation of BER intermediate substrates, indicating that balanced levels of active BER proteins are critical for genome maintenance. Our results suggest that aberrant BER drives carcinogenesis. Using high throughput screening technologies, we have also found that specific BER variants confer either sensitivity or resistance to various cancer therapies. Mechanisms underpinning the BER variant responses to cancer therapies will be discussed.

**S-05.01.1-002****Inhibition of DNA repair proteins via small molecule compounds as potential drugs in cancer therapy****M. Dizdaroglu<sup>1</sup>**, A. C. Jacobs<sup>2</sup>, N. Donley<sup>2</sup>, M. J. Calkins<sup>2</sup>, A. Jadhav<sup>3</sup>, D. Dorjsuren<sup>3</sup>, D. Maloney<sup>3</sup>, A. Simeonov<sup>3</sup>, P. Jaruga<sup>1</sup>, E. Coskun<sup>1</sup>, A. K. McCullough<sup>2</sup>, R. S. Lloyd<sup>2</sup><sup>1</sup>*National Institute of Standards and Technology, Gaithersburg, Maryland, United States of America*, <sup>2</sup>*Oregon Health and Science University, Portland, Oregon, United States of America*, <sup>3</sup>*National Institutes of Health, Rockville, Maryland, United States of America*

Many anticancer agents kill cancer cells by damaging their DNA. One important mechanism by which cancer cells develop resistance to therapy is to increase their DNA repair capacity. This is achieved in part by overexpressing DNA repair proteins that remove DNA lesions before they become toxic. Thus, DNA repair pathways are promising therapeutic targets for novel cancer treatments. Efforts are underway worldwide to find inhibitors of DNA repair proteins. Most targeted proteins belong to the base excision repair pathway. However, the development of inhibitors has been lagging for DNA glycosylases, which remove modified DNA bases in the first step of this pathway. Recently, several DNA glycosylases were identified as potential targets in combination therapeutic strategies. We chose NEIL1 as the proof-of-principle DNA glycosylase to design experiments to discover small molecule inhibitors. To detect both glycosylase and lyase activities of NEIL1, a fluorescence-based assay was developed. Small molecule compound libraries were screened to find inhibitors of NEIL1. A number of purine analogs were found that fit with the paradigm of NEIL1 action on damaged purines. Inhibitors of other DNA glycosylases OGG1 and NTH1 were also found. In addition, we applied mass spectrometry to measure the effect of the inhibitors on glycosylase activities. The data showed significant differences in inhibition of enzymatic activities among these DNA glycosylases. Purine analogs inhibited NEIL1 and NTH1 by mainly blocking the glycosylase activity. They likely fit inside the active site hindering the residues required for activity. Hydrazide inhibitors of OGG1 blocked Schiff base formation, indicating that they mainly function by inhibiting the combined glycosylase/lyase activity. Overall, this work forms the foundation for the future discovery of DNA repair inhibitors as anticancer drugs for the entire family of DNA glycosylases.

**ML-05.01.1-001****Conformational gating as a strategy for damaged base discrimination by DNA repair enzymes****A. Popov<sup>1</sup>**, A. Endutkin<sup>1</sup>, **D. Zharkov<sup>1,2</sup>**<sup>1</sup>*SB RAS Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*, <sup>2</sup>*Novosibirsk State University, Novosibirsk, Russia*

To avoid mutagenic and carcinogenic load by environmentally and internally generated DNA lesions, living cells remove damaged bases through the pathway known as base excision repair. Although the structures of many repair enzymes have been solved, it is still unclear how they achieve their selectivity, since usually the lesion-specific bonds formed in the active site cannot provide sufficient power to discriminate damaged bases against a  $\sim 10^6$ – $10^7$  excess of normal ones. We have combined molecular dynamics, stopped-flow enzyme kinetics, NMR, and thermodynamic measurements to analyze how structural and energetic

features of damaged bases could be used for their recognition. In the most informative system so far, the oxidized base 8-oxoguanine (oG), recognized by enzymes of three structurally unrelated folds, was found by NMR to affect the DNA dynamics and change the backbone conformation. Melting profiles showed that oG differed in stacking energy from normal G, and time-resolved spectroscopy data were indicative of facilitated duplex opening. Thus, 8-oxoG forms a “soft spot” in DNA that could serve as the major early recognition feature. Steered molecular dynamics with umbrella sampling indicated that eversion of oG into the active site of two DNA glycosylases, *E. coli* Fpg and human OGG1, proceeds through several conformational intermediates, some of which are selective towards oG thus serving as kinetic gates. Disruption of these transient interactions abrogated the enzyme activity *in vitro*. Several other enzymes specific for modified DNA, including mismatched thymine–DNA glycosylase (TDG) and 5-methylcytosine dioxygenase were also investigated by molecular dynamics, revealing that conformational gating might be a common strategy in selective recognition of rare DNA bases.

This work was supported by RSF (14-24-00093) and RFBR (14-04-01879).

### ML-05.01.1-002

#### Nucleotide excision repair by dual incisions in plants

F. Cantürk<sup>1,2</sup>, M. Karaman<sup>2,3</sup>, C. P. Selby<sup>2</sup>, M. G. Kemp<sup>2</sup>, G. Kulaksiz-Erkmen<sup>2,4</sup>, J. Hu<sup>2</sup>, W. Lia<sup>2</sup>, L. A. Lindsey-Boltz<sup>2</sup>, A. Sancar<sup>2</sup>

<sup>1</sup>Erciyes University, Kayseri, Turkey, <sup>2</sup>University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States of America, <sup>3</sup>Kilis 7 Aralık Ün., Kilis, Turkey, <sup>4</sup>Hacettepe University, Ankara, Turkey

This aim of study was to investigate nucleotide excision repair mechanism in plants. Plants use light for photosynthesis and for various signaling purposes. The UV wavelengths in sunlight also introduce DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts [(6-4)PPs] that must be repaired for the survival of the plant. Genome sequencing has revealed the presence of genes for both CPD and (6-4)PP photolyases, as well as genes for nucleotide excision repair in plants, such as Arabidopsis and rice. The *A. thaliana* ecotype Columbia cell line T87 was obtained from the Arabidopsis Biological Resource Center at The Ohio State University. UV irradiation and lysate preparation steps were performed in a dark room with dim yellow light illumination. Cells were exposed to 50 J·m<sup>-2</sup> of UV-C. Following repair, we were used immunoslot blot analysis and *in vivo* excision repair assay.

Plant photolyases have been purified, characterized, and have been shown to play an important role in plant survival. In contrast, even though nucleotide excision repair gene homologs have been found in plants, the mechanism of nucleotide excision repair has not been investigated. Here we used the *in vivo* excision repair assay developed in our laboratory to demonstrate that Arabidopsis removes CPDs and (6-4)PPs by a dual-incision mechanism that is essentially identical to the mechanism of dual incisions in humans and other eukaryotes, in which oligonucleotides with a mean length of 26–27 nucleotides are removed by incising ~20 phosphodiester bonds 5' and 5 phosphodiester bonds 3' to the photoproduct.

### Sunday 4 September

15:30–17:30, Hall A

#### Developmental biology

### S-02.09.1-001

#### Evolution of color and motion vision

C. Desplan

NYU, New York, United States of America

How do changes in cell fate affect the function and performance of neural systems, in particular to adapt visual systems to their environment? For example, butterflies have improved color vision compared to other insects such as *Drosophila*. I will discuss how this is achieved by duplicating the (R7) photoreceptor cell: Each of the two R7s makes a stochastic choice to express the transcription factor Spineless, leading to three stochastic types of ommatidia (Spineless on/on, on/off or off/off), instead of the two found in *Drosophila* (Spineless on or off). We used CRISPR to decipher the function of Spineless in butterflies and showed that it controls the fate of the ommatidia. This shows that evolution can shape the visual system to adapt it to specific environmental conditions by modifying a more ancestral gene network that determines photoreceptor fate.

The opposite change occurs in the housefly *Musca* that has improved motion vision at the expense of color vision, again through changes in specification of cell fate. In a region of the male eye that is dedicated to chasing females (the “love spot”), R7 color photoreceptors express the broad spectrum rhodopsin normally used in motion vision. They are also rewired to connect to the motion processing centers for additional sensitivity. I will discuss the molecular basis for this fate transformation and modification of axon targeting.

Using insect eyes, we can show how developmental systems can be modified for specific function and how the incredible diversity of neural fates found in the visual system are properly specified during development.

### S-02.09.1-002

#### The function of signalling oscillations during mouse embryo mesoderm patterning

K. Sonnen, A. Aulehla

European Molecular Biology Laboratory, Heidelberg, Germany

We are studying the temporal aspect, or timing, of embryonic development and in particular, investigate the role of embryonic clocks, or oscillators. Oscillations in Notch-, Wnt- and Fgf-signaling pathway activity (period ~2 h) have been identified during mesoderm segmentation in mouse embryos and are linked to the periodic formation of pre-vertebrae, the somites. Most strikingly, oscillations occur phase-shifted between neighboring cells, producing spatio-temporal wave patterns within the embryonic mesoderm. Combining real-time imaging of customized dynamic reporter mouse lines with functional perturbations and also novel *ex vivo* models for mesoderm patterning, we will present our latest findings addressing the role of spatiotemporal signaling oscillations during mesoderm patterning and cellular differentiation.



**ML-02.09.1-001****The effect of nitric oxide inhibition on chick embryo and liver development**

F. Cöllü, B. Gürcü

*Department of Biology, Zoology Section, Faculty of Science and Letters, Celal Bayar University, Manisa, Turkey*

Nitric oxide (NO) is an inorganic free radical that secreting primarily from the epithelial cells and have roles on physiology events like defense against the microorganisms. L-Nitro-Arginine-Methyl-Ester (L-NAME) can act a NOS substrate agent, and therefore, a non-selective inhibitory for using the inhibition of both endothelial NO and inducible NO synthesis both *in vivo* and *in vitro* with non-specific matter.

The purpose of our study is detecting structural anomalies on chick embryo and liver development in correlation with completely inhibiting or diminishing of NO synthesis simultaneously.

In our study, Leghorn type embryonic chick eggs has been used. Eggs of both sham and experiment group has been incubated in a condition that have  $37 \pm 0.5^\circ\text{C}$  heat and  $60 \pm 0.5\%$  moisture level. At experiment group embryos, L-NAME (SCBT- sc-200333A) at two different doses ( $15\text{--}30 \text{ mg kg}^{-1}$ ) in egg yolk at 4th, 5th, 6th and 7th days. Twenty-four hour after the application, embryos were fixated and livers were used for routine histology.

At embryos, morphologically; developmental latency, shrinkage at the face, disruption flattening at the face contours, heart and extremity deformations, disruption at the brain vesicle and reduction at the eye pigmentation has been observed. Meanwhile at the liver tissue, histologically; increasing of mitotic cells, malfunction at the cordon forming capabilities of hepatocytes, expansion at the sinusoids, endothelial cell loss, and stacking of erythrocyte clusters at sinusoids and necrotic areas has been observed.

At the embryonic development process, NO has positive effects on embryos, and in case of NO inhibitor agent presence, decreased NO has general effects on embryonic morphology and can induce histopathologic changes especially on liver. With this status, it is concluded that NO inhibition might be involved negatively with lifespan and life quality in embryo development.

**ML-02.09.1-002****Association between the TGFb3 signaling cascade and the IRF6/DNp63 genes in cleft palate**F. Ozturk<sup>1,2</sup>, A. Nawshad<sup>2</sup><sup>1</sup>*Canik Basari University, Samsun, Turkey,* <sup>2</sup>*University of Nebraska Medical Center, Lincoln, NE, United States of America*

Cleft palate (CP) is the second most common birth defect (1/800 live births) in humans, and is caused by the lack of fusion of the embryonic palatal shelves early in gestation (6–10 weeks). The formation of a continuous palate is a complex process involving multiple steps, including: palatal shelf growth, elevation, attachment, and fusion. The stages of palatogenesis are regulated by numerous genes that are essential for normal palate development, thus, the cleft palate has been considered as a multigenic disorder. Interestingly, murine models of knockout ( $-/-$ ) TGFb3, interferon regulatory factor 6 (IRF6) ( $-/-$ ), and truncated p63 (DNp63) ( $-/-$ ) are born with palatal clefts because of failure of the palatal shelves to adhere, suggesting that these genes regulate palatal epithelial differentiation. However, despite having similar phenotypes in null mouse models, no studies have analyzed the possible association between the TGFb3 signaling cascade and the IRF6/DNp63 genes during palate development. Our study

analyzes the regulatory role of TGFb3, DNp63, and IRF6 on the desquamation of periderm prior to contact of the palatal shelves.

We performed biochemical analysis, SEM imaging, gene activity and protein expression assays with palatal sections of TGFb3 ( $-/-$ ), DNp63 ( $-/-$ ), and wild-type (WT) embryos, and primary MEE cells from WT palates to uncover the association between TGFb3 and IRF6/DNp63.

Our results suggest that periderm degeneration depends on functional TGFb3 signaling to repress DNp63, thereby coordinating periderm desquamation. Cleft palate occurs in TGFb3 ( $-/-$ ) because of inadequate periderm removal that impedes palatal seam formation, while cleft palate occurs in DNp63 ( $-/-$ ) palates because of premature fusion.

We concluded that IRF6 and DNp63 are essential for palatal epithelium, and the generation and maintenance of the periderm. However, IRF6 is not regulated by TGFb3 and/or by DNp63, and it may not facilitate periderm desquamation directly.

**Sunday 4 September****15:30–17:30, Hall B****New optical methods for studying neuronal structure and function****S-09.01.1-002****Single-molecule mapping the transcellular architecture that regulates synaptic transmission**

T. Blanpied

*University of Maryland School of Medicine, Baltimore, United States of America*

Synaptic transmission in the brain is maintained by a delicate, subsynaptic molecular architecture, and even mild alterations in synapse structure drive functional changes during learning and disease. Key to this architecture is how the distribution of vesicle fusion sites in the active zone (AZ) of the presynaptic cell corresponds to the position of neurotransmitter receptors in the postsynaptic density (PSD) of the postsynaptic cell. However, the submicron size of synapses has precluded examination of the key mechanisms underlying the spatial organization of the AZ and those at play within the PSD that control receptor positioning.

To address the synapse structure-function relationship at nanoscale resolution in live cells, we have developed a series of functional approaches for concurrent use with single-molecule localization microscopy (PALM and STORM). We find that RIM and other key proteins regulating vesicle fusion are mutually co-enriched within nanometer-scale subregions of the AZ. This organization impacts synapse function in many ways. First, by mapping sites of single-vesicle fusion events within individual AZs, we revealed that evoked fusion occurs in a confined subregion of the AZ where RIM density is highest. Second, the distributions of RIM and receptors are highly co-aligned across the synaptic cleft. Third, concurrent PALM and GCaMP6f imaging in single synapses allows us to examine how postsynaptic nanostructure controls NMDA receptor activation. Finally, within the PSD, single-molecule tracking and FRAP reveal that the pattern of receptor positions is controlled by a combination of receptor binding and macromolecular crowding. Together, combining functional and super-resolution imaging reveals that the nanoarchitecture of the active zone directs vesicle fusion to occur preferentially at sites nearest postsynaptic receptor ensembles. This provides a simple organizational principle by which CNS synapses maintain and modulate synaptic efficiency.

**S-09.01.1-001****Monitoring and manipulating intracellular transport in living neurons**

C. Hoogenraad

*Cell Biology, Faculty of Science, Utrecht University, Utrecht, the Netherlands*

Controlling protein-protein interactions in live cells represents a powerful tool in modern biology and has opened up new avenues for manipulating cellular processes. Chemical and light induced dimerization systems allow spatially and temporally control of transcriptional activation, signal transduction pathways, subcellular protein translocations and other cellular processes. We have currently developed inducible cargo transport assays to study the basic trafficking rules in neurons. By recruiting specific motor proteins (kinesin, dynein or myosin) to selected organelles (e.g. synaptic vesicles, mitochondria or RNA particles), these organelles will be forced to move anterogradely, retrogradely or become immobilized. Because these approaches allow spatiotemporally controlled removal and positioning of selected organelles, they will be invaluable tools to unravel their local functions in developing and mature neurons. Here we will discuss recent advances in engineering inducible tools and discusses future directions to monitor and manipulate intracellular transport processes in living neurons *in vitro* and *in vivo*.

**S-09.01.1-003*****In vivo* imaging of axon degeneration**

T. Misgeld

*Technical University of Munich, München, Germany*

In my talk I will discuss how structural and functional *in vivo* imaging in transgenic mice can be used to analyze the cell biological mechanisms underlying axon dismantling. Specifically I will present data on how assays of organelle dynamics and function can be applied to settings of axon dismantling in development and disease.

**ML-09.01.1-001****Pgp expression in mice brain tissue after the administration of some CNS active drugs**A. C. Nicolae<sup>1</sup>, C. M. Dragoi<sup>1</sup>, V. Vuta<sup>2</sup>, G. T. A. Burcea-Dragomiroiu<sup>3</sup>, D. E. Popa<sup>3</sup>, I. Dumitrescu<sup>4</sup>, A. L. Arsene<sup>5</sup><sup>1</sup>*Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania,*<sup>2</sup>*Virology Department, Institute for Diagnosis and Animal Health, Bucharest, Romania,*<sup>3</sup>*Department of Drug Control, Faculty of Pharmacy, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania,*<sup>4</sup>*Department of Pharmaceutical Physics and Informatics, Faculty of Pharmacy, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania,*<sup>5</sup>*Department of Microbiology, Faculty of Pharmacy, University of Medicine and Pharmacy Carol Davila, Bucharest, Romania*

A highly sensitive and reliable method to determine the expression of P-glycoprotein (Pgp) is quantitative immunofluorescence using specific antibodies. Thus, Pgp has a role in assessing the distribution of drugs in the brain, the efflux transporter that limits drug penetration into brain tissue through the blood-brain barrier.

Our objective was to determine the Pgp expression *in vivo* by using a small animals experimental model (white Albino Swiss mice) randomly divided into 24 groups (10 animals/group). After the administrations of central nervous system active drugs (valproic acid, risperidone, thioridazine, fluoxetine, lithium, as

well as combinations of these drugs), we determined the expression of Pgp by an indirect quantitative immunofluorescence method.

The experimental study was conducted by quantifying the Pgp expression in mice brain tissue. On fresh brain samples there were performed imprints on microscope slides, fixed and analyzed according to the method of indirect immunofluorescence.

The examination was conducted using a DMIL Leica fluorescence microscope, EBQ 100 Isolated, UV, B. Images were obtained with Nikon D40 and processed with ImageJ software.

Our results revealed significant changes in the expression of Pgp in the brain tissue, denoting the inhibition of the efflux pump. From this point of view, the most potent *in vivo* inhibitor of the studied drugs was found to be risperidone.

An absolute novelty for current research in the pharmacotherapy field, is the synergistic *in vivo* potentiation of the inhibitory effect on the expression of Pgp at the central nervous system level, revealed by two of the studied drugs: fluoxetine and valproic acid.

Of all five studied drugs (valproic acid, risperidone, thioridazine, fluoxetine, lithium), lithium showed the strongest effect on the expression of Pgp compared to the control.

**Monday 5 September****9:00–11:00, Hall A****RNA biology, biogenesis and processing****S-01.02.2-001****Long non-coding RNAs – messages from the dark matter of the lung cancer genome**S. Diederichs<sup>1,2</sup><sup>1</sup>*University of Freiburg, Freiburg, Germany,* <sup>2</sup>*German Cancer Research Center (DKFZ), Heidelberg, Germany*

Non-coding RNA profiles in cancer are largely unknown which greatly impedes the discovery of functionally important ncRNAs in tumorigenesis as well as the generation of genome-wide libraries.

The long non-coding RNA MALAT1 was one of the first lncRNAs associated with cancer: it is a highly conserved nuclear ncRNA and a predictive marker for metastasis development in lung cancer. However, its high abundance and nuclear localization have greatly hampered its functional analysis since it is only inefficiently knocked down by RNA interference (RNAi).

To uncover its functional importance, we developed a MALAT1 knockout model in human lung tumor cells by genomically integrating RNA destabilizing elements site-specifically into the MALAT1 locus using Zinc Finger Nucleases (ZFN). This approach yielded a more than 1000-fold silencing of MALAT1 providing a unique loss-of-function model.

Proposed mechanisms of action of MALAT1 include regulation of splicing or gene expression. In lung cancer, MALAT1 does not alter alternative splicing but actively regulates gene expression inducing a signature of metastasis-associated genes. Consequently, MALAT1-deficient cells are impaired in migration and form fewer tumor nodules in a mouse xenograft model.

Encouraged by this discovery of the essential function of MALAT1 in lung cancer metastasis, we wanted to analyze whether MALAT1 could also be therapeutically targeted: We developed Antisense oligonucleotides (ASOs) effectively blocking MALAT1 expression in the cell culture and in the animal. Notably, MALAT1-ASO treatment prevents metastasis formation after tumor implantation. Thus, targeting MALAT1 with antisense oligonucleotides provides a potential therapeutic approach

to prevent lung cancer metastasis with MALAT1 serving as both, predictive marker and therapeutic target.

Lastly, regulating gene expression, but not alternative splicing is the critical function of MALAT1 in lung cancer metastasis.

### S-01.02.2-003

#### Small non-coding RNA host genes in cancer

A. H. Lund

*University of Copenhagen, Copenhagen, Denmark*

Long non-coding RNAs (lncRNAs) are emerging as important players in many aspects of cellular biology as well as in human pathologies. To identify lncRNAs with important functions in cancer, we recently performed a large-scale profiling study of non-coding RNAs in 5 cancer types and several cancer-related cell culture model systems.

Interestingly, among the most de-regulated transcripts we found several host genes for small RNAs, such as snoRNAs and microRNAs. Whereas such host genes have previously been described as short-lived and biologically inert carriers, we find that several of them have important functions in cancer-related processes such as senescence and apoptosis. In this lecture I will provide examples of small non-coding RNA host gene lncRNAs with important functions in cancer with special emphasis on their molecular mechanism of action.

### S-01.02.2-002

#### lncRNAs as functional molecules in cancer pathways

M. Huarte

*Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain*

Cellular networks are fine-tuned and maintained by the coordinated function of not only proteins, but also non-coding RNAs (ncRNAs). In addition to the well-characterized protein-coding constituents, large noncoding RNAs are emerging as important regulatory molecules in tumor-suppressor and oncogenic pathways. Supporting this idea, we have found that the transcription factor p53, which is crucial for the maintenance of cellular homeostasis, specifically regulates the expression of dozens of long noncoding RNA genes (lncRNAs). These lncRNAs are bona-fide transcriptional targets of p53, and are induced by p53 to modulate specific facets of the p53 cellular response, including the regulation of gene expression through epigenetic mechanisms. Additionally, we found that p53 is involved in the repression of lncRNAs with oncogenic functions, which are required for uncontrolled cell proliferation and are overexpressed in multiple tumor types. Altogether, our work suggests that large non-coding RNAs constitute an unknown layer of regulation of the p53 cellular response that could represent future novel targets for cancer treatments.

### ML-01.02.2-001

#### Dynamic methylation of mRNA during early development

A. Klungland, J.A. Dahl, E. A. Alemu, H. Aanes

*Oslo University Hospital, Oslo, Norway*

A broad repertoire of modifications is known to underlie adaptable coding and structural function of proteins, DNA and various RNA species. Methylations of mammalian DNA and histone residues are known to regulate transcription and the discoveries of demethylases that remove methylation in DNA and

histones provide a basis for the understanding of dynamic regulation of mammalian gene expression. The reversions of methyl marks on DNA and proteins have been extensively studied the last decade. On the contrary, reversal of N6-methyladenosine (m6A) to adenosine (A) in messenger RNA (mRNA) was only identified recently. 6-methyladenine (m6A) is the most abundant internal base modification of messenger RNA (mRNA) in higher eukaryotes. Together with our collaborators, we have identified a m6A demethylase for mRNA (Zheng et al., *Molecular Cell* 2013) and developed technology for single-base resolution mapping of m6A in mRNA (Ke et al., *Genes Dev* 2015). Internal m6A is the most common modification of mRNA in higher eukaryotes. Male mice lacking Alkbh5 have elevated m6A levels in total mRNA and are characterized by impaired fertility resulting from apoptosis that affects meiotic metaphase-stage spermatocytes. The discovery of this RNA demethylase strongly suggests that the reversible m6A modification has fundamental and broad functions in mammalian cells and in human disease. We currently investigate the dynamics of m6A during early development.

### ML-01.02.2-002

#### What are the key aspects of interaction between RNA polymerase II C-terminal domain phosphorylated on tyrosine-1 and the elongation factors

K. Kubicek<sup>1</sup>, M. Krejcikova<sup>1</sup>, P. Brazda<sup>1</sup>, E. Smirakova<sup>1</sup>, J. Novacek<sup>1</sup>, P. Cramer<sup>2</sup>, R. Stefl<sup>1</sup>

<sup>1</sup>*Central European Institute of Technology, Masaryk University, Brno, Czech Republic.* <sup>2</sup>*Gene Center and Department of Biochemistry, Ludwig-Maximilians-Universität München, Munich, Germany*

Post-translational modifications of the consensus motif Y1-S2-P3-T4-S5-P6-S7 of the C-terminal domain (CTD) of RNA polymerase II are since last decade known as the “CTD code”. These modifications are dynamic and specific for each phase of the transcriptional cycle. Increased phosphorylation levels of Y1 during the productive elongation prevents binding of termination factors, and stimulates recruitment of elongation factors. However, there is no structural information on how the phosphotyrosine modified CTD is recognized by these elongation factors.

To investigate phosphotyrosine recognition, we employed integrative approach to structural biology – namely combination of solution nuclear magnetic resonance, small angle X-ray scattering, mass spectrometry and X-ray crystallography, supported by functional studies with point/multiple mutations. We will present the structural data for phosphotyrosine recognition within the CTD by the elongation factor, which help to decipher how this important CTD modification mark is read out by transcription factors. The results of this research have been acquired within CEITEC 2020 (LQ1601) project with financial contribution made by the Ministry of Education, Youths and Sports of the Czech Republic within special support paid from the National Programme for Sustainability II funds.

**Monday 5 September**  
**9:00–11:00, Hall B**

**Proteins in action**

**S-02.02.2-001**

**The mechanism of dynein motility**

A. Yildiz

*University of California Berkeley, Berkeley, United States of America*

My laboratory has made significant contributions in understanding the molecular mechanism of dyneins, biological nanomachines responsible for the transport of a wide variety of cargoes along microtubule filaments. We showed that, unlike kinesin and myosin motors, dynein moves processively without coordination between its catalytic domains and follows a helical trajectory around microtubules. The mechanism of dynein directionality is also distinct from other motors and is a result of unique mechanical and structural features of the catalytic domain. We have also identified an ATPase site that repurposes dynein for microtubule anchoring and cargo transportation functions inside cells. These studies present a robust mechanistic model of processivity and force generation that has altered the established views regarding how motors transport intracellular cargoes over long distances.

**S-02.02.2-003**

**Ultrahigh resolution fluorescence-force single molecule spectroscopy and engineering of a superhelicase**

T. Ha<sup>1,2</sup>

*<sup>1</sup>Departments of Biophysics and Biophysical Chemistry, Biophysics and Biomedical Engineering, Johns Hopkins University, Baltimore, United States of America, <sup>2</sup>Howard Hughes Medical Institute, Baltimore, United States of America*

Double stranded needs to be separated into single strands by helicase enzymes for genome duplication or repair to occur. Defects in helicases or their mis-regulation can cause serious human genetic diseases including cancer and premature aging. If helicases unwind every nucleic acids they encounter, unregulated generation of single strands can be toxic to the cell. How is the unwinding activity regulated? Can we unleash potent helicase activities that are normally suppressed based on our understanding of helicase regulation? In an attempt to identify the functionally active conformation, we discovered that *E. coli* Rep helicase, once intramolecularly crosslinked into the closed conformation, termed Rep-X, becomes a superhelicase that unwinds thousands of bp without falling off even against strong opposing forces. In contrast, a wild type Rep monomer has undetectable unwinding activity. A related helicase PcrA could also be converted to a superhelicase PcrA-X through crosslinking into the closed conformation, and its partner protein known to activate unwinding stabilizes the closed conformation, suggesting a plausible mechanism of turning on the unwinding activity *in vivo*. Why did then Nature create the other conformation, i.e. open conformation? Using ultrahigh resolution optical trap combined with single fluorophore sensitivity and doubly labeled UvrD to distinguish between different conformations through FRET, we showed that a related helicase UvrD adopts a closed form during unwinding but after unwinding about a dozen basepairs it switches to the open conformation and reverse direction to allow DNA re-zipping in its wake. Rep-X or PcrA-X therefore becomes a superhelicase because the open conformation, required to change direction, is inaccessible. Overall, our studies revealed an entirely novel

mechanism of helicase regulation and led to the engineering of a superhelicase with potential biotechnological and biophysical applications.

**S-02.02.2-002**

**Non-equilibrium steady state transitions in a model actin cortex**

T. Tan<sup>1</sup>, M. Malik Garb<sup>2</sup>, E. Abu-Shah<sup>2</sup>, J. Li<sup>1</sup>, A. Sharma<sup>3</sup>, F. McKintosh<sup>4</sup>, K. Keren<sup>2</sup>, N. Fakhri<sup>1</sup>, C. Schmidt<sup>3</sup>

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Thermodynamic non-equilibrium is a defining feature of living systems on all levels of organization. Cells and tissues are built of “active matter”, dynamic materials with built-in force generators. Such materials self-organize in biological systems into well-ordered dynamic steady states, sustained by the dissipation of metabolic energy. The materials show striking collective phenomena on a mesoscopic scale, reminiscent of second order phase transitions and criticality.

We have used advanced light microscopy and single particle tracking of IR fluorescent single-walled carbon nanotubes to characterize motion and stress patterns in a steady state actin cortex model system. We find intriguing transitions between distinct dynamic steady states when we change crosslinker concentration in the system and drive the cortex through a mechanical percolation transition.

**ML-02.02.2-001**

**Dynamics and interactions in a 1MDa chaperonin at atomic resolution**

J. Guan, E. Colas-Debeld, G. Mas, P. Macek, E. Crublet, C. Moriscot, G. Schoehn, P. Gans, P. Schanda, J. Boisbouvier

*Institute de Biologie Structurale, CNRS/CEA/UGA, Grenoble, France*

Chaperonins are huge ATP-dependent chaperones (1 MDa) that are essential for proper cellular protein folding, and have shown links to many aging-associated diseases. Misfolded proteins are isolated and refolded in chaperonins and finally released. Atomic details of the mechanism of these nanomachines remain limited due to the size and the dynamic feature of the complexes.

Nuclear magnetic resonance (NMR) spectroscopy offers a unique ability to monitor structural and dynamic changes at atomic resolution. Recent advances in specific isotope labeling of protein methyl groups and in proton-detected, solid-state magic angle spinning have significantly extended the frontier of NMR for challenging biomolecules. We use a combination of advanced isotope labeling, paramagnetic tagging, solution- and solid-state NMR integrated with electron microscopy (EM) and mass spectrometry (MS), to probe the modes of actions of a 1 MDa chaperonin, along with different sizes of substrates.

We observe the structural rearrangement corresponding to the different states during the functional cycle of this large biological machinery processing its substrates at atomic resolution. Complementary biochemical assays on the protein complexes showed the chaperonin indeed prevents aggregation of the substrates. Interestingly, we observed direct evidence for the unfoldase activity of chaperonins in absence of ATP, and characterized the dynamical state of proteins encapsulated in the folding chamber. Binding of ATP triggers the refolding and release of the substrate protein inside the chaperonin.

We demonstrate that the interaction between the chaperonin and different substrate proteins can be studied by a combined approach using NMR, EM, MS, and biochemical experiments. This combined approach allows us to characterize MDa-large functional protein complexes in a time- and atomic-resolved manner. This is also the first solid-state NMR study of a substrate protein inside a chaperonin.

### ML-02.02.2-002

#### Dynamic interaction of Elk-1 with motor proteins and mitotic kinases

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Gebze Technical University, Kocaeli, Turkey

**Introduction:** Elk-1 is a transcription factor of the ETS domain superfamily, known to be activated by the ERK MAPK pathway in response to mitogens, yet is also known to be present in the axons and dendrites of post-mitotic neurons. It has previously been shown in our laboratory to colocalize to mitotic spindle poles of brain tumor cells during mitosis, and translocate to the midzone followed by spindle midbody during cytokinesis. In order to understand how this translocation occurs, we have carried out a series of biochemical and bioinformatics analyses.

**Materials and Methods:** SH-SY5Y and U87 cell lines were transfected with pCDNA3- or pCMV-based constructs using PEI reagent. Fluorescent confocal microscopy, GST pulldown and co-IP experiments were used to study protein-protein interactions. Site-directed mutagenesis and deletions were used to determine sites of interaction. FACS analysis was used to confirm nocodazole-synchronization of cell lines.

**Results:** We and others had previously shown that in neurons Elk-1 in axons retrogradely translocated to the nucleus upon stimulation. To understand how this translocation occurs, we have analyzed its colocalization and interaction with major motor proteins. We have shown that Elk-1 not only colocalized with kinesin during mitosis, but also showed an unexpected localization to mitotic spindles from metaphase to cytokinesis. We have shown that serum stimulation was important for interaction of Elk-1 with dynein, however S383 phosphorylation did not seem to be a factor in this interaction. Upon phosphorylation prediction analyses, various putative Plk, Aurora kinase and Cdk phosphorylation motifs were identified on Elk-1, and Elk-1 indeed does interact with these kinases in mitosis.

**Discussion and Conclusion:** Our results reveal a novel and interesting interaction of a transcription factor with various cell cycle-related kinases, and its dynamic localization on the mitotic apparatus during mitosis.

Monday 5 September  
9:00–11:00, Hall C

### Computational biology

#### S-03.02.2-001

#### Approaching IDP function by dynamic structural ensembles

P. Tompa

VIB Structural Biology Research Center, Brussels, Belgium

Intrinsically disordered proteins (IDPs) and complex multidomain proteins are characterized by a dynamic ensemble of conformations that cannot be unequivocally described by traditional static terms of structural biology (1). Their structural ensemble is dynamic and malleable, enabling them to adapt to a wide variety

of regulatory signals. The quantitative description of structural ensembles has just started (2) and here we will show that it holds the promise to elucidate complex protein regulatory phenomena (3), such as moonlighting (4) and allostery (5) in the “supertertiary” structure (6) of proteins.

(1) Tompa, P. (2011) Unstructural biology coming of age. *Curr. Opin. Struct. Biol.* 21: 419.

(2) Varadi, M. et al. (2014) pE-DB, a database of protein structural ensembles. *Nucl. Acids. Res.* 42: D326.

(3) Tompa, P., Varadi, M. (2014) Predicting the predictive power of IDP ensembles. *Structure* 22: 177.

(4) Tompa, P., Szász, Cs. and Buday, L. (2005) Structural disorder throws new light on moonlighting. *Trends Biochem. Sci.* 30, 484–489.

(5) Tompa, P. (2014) Multiteric regulation by structural disorder in modular signaling proteins: an extension of the concept of allostery. *Chem. Rev.* 114, 6715.

(6) Tompa, P. (2012) On the supertertiary structure of proteins. *Nature Chem. Biol.* 18, 597.

#### S-03.02.2-002

#### The versatile roles of peptide-mediated interactions detected by modeling and experiment

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Peptide-mediated interactions play major roles in the regulation of the cell, and it is therefore important to be able to model and manipulate the structure and function of these interactions. I will in my talk provide a short overview of our work on the modeling and characterization peptide-mediated interactions, including our Rosetta-FlexPepDock high-resolution protocol for peptide docking and its implementation for binding prediction, FlexPepBind. I will then present more recent work on the extension towards global docking of peptides, highlighting insights about peptide-protein association as well as challenges ahead.

Peptide-mediated interactions usually do not come alone – it is their integration that allows for the detailed regulation of crucial processes. I will end my presentation with the description of different strategies that are used to integrate information from different peptide binding events.

#### S-03.02.2-003

#### Personalized health – harnessing the power of diversity

B. Rost

Technical University of Munich, Munich, Germany

The objective of our group is to predict aspects of protein function and structure from sequence. The wealth of evolutionary information available through comparing the whole bio-diversity of species makes such an ambitious goal achievable. Our particular niche is the combination of evolutionary information with machine learning. We developed methods to predict from sequence protein interactions (incl. networks), cellular localization, functional classifications and the effects of sequence variants upon molecular function and the organism. In this talk I will focus on protein-protein interactions, present the concept of the Dark Proteome and how protein disorder appears to play a unique role in evolution, and will present some surprising results from effect-prediction methods for the analysis of large populations.

**ML-03.02.2-001****Steered molecular dynamics analysis of four substrates of SPOP: Ci, Daxx, MacroH2, and Puc****Z. Emami***Koç Üniversitesi Koç Üniversitesi, İstanbul, Turkey*

The significance of Speckle-Type Poz Protein (SPOP) in the pathway of prostate cancer is evident, as it has the highest rate of occurrence among the point mutation induced carcinomas. Furthermore, malfunction of SPOP has been shown to be involved in several another types of cancers as colorectal, endometrial, liver and kidney cancers as well.

SPOP is a member of the MATH-BTB proteins, with the main character of having the two protein domains of MATH and BTB. MATH-BTB proteins are encoded by 131 genomes and function in protein degradation. Cullin-based E3 complexes accompanied by a substrate adaptor, ubiquitinate their substrate proteins. For Cullin3, the substrate adaptor is SPOP. SPOP identifies and bind its substrates through its MATH domain. All the tumorigenic mutations in SPOP have been reported to lie in the MATH domain. The five aromatic residues of Y87, F102, Y123, W131 and W133, in MATH domain have the most involvement in the binding of SPOP to the substrate. Interestingly Y87A, Y123A, W131A, F133A and D130A mutations, all have been shown to be strongly deleterious in substrate binding. A linear motif structure of  $\Omega$ - $\pi$ -S-S/T-S/T ( $\Omega$  stands for nonpolar and  $\pi$  for polar) is found in most of the substrates of SPOP. The variability in the four of the five residues, tunes the degree of affinity among SPOP and the substrate, as SPOP has a variety type of substrates, each of which should be degraded to a suitable degree.

SPOP has a range of substrates including Puc, MacroH2, Daxx, and Ci. In this study, we use the MATH domain part of the 3HQI PDB entry as the basic frame of the calculations for each of the substrates. We use Steered Molecular dynamics simulations for each motif and calculate the binding affinity ( $K_d$ ) from plots of work against time for each of the substrates. Verifying the experimental data for the  $K_d$  will be an introduction to more insight to SPOP and its pathology.

**ML-03.02.2-002****Disruptive mutations elucidated by centrality of residues and redundancies in inter-residue communication pathways****T. F. Guclu, C. Atilgan, A. R. Atilgan***Sabancı University, İstanbul, Turkey*

Dynamics of a protein may be changed by point mutations that lead to drastic or null differences in the function. The correspondence of mutations and function modification determines the evolutionary consequence of the local change. To relate point mutations to function, we study alterations in the communication within the residue network of a protein by performing computational alanine scanning on a selected non-homologous set of 350 proteins.

The scheme is based on mutating each amino acid to alanine, and minimizing in water at isotonic salt concentration at the all-atom resolution. We then assign a node to each residue and links between directly coordinating node-pairs, treating the protein as a graph. We focus on changes in shortest path length of residues ( $\Delta L$ ) and betweenness centrality ( $\Delta BC$ ) of mutated nodes with respect to the wild-type, averaged over all mutations.

As a case study, bacterial enzyme dihydrofolate reductase (DHFR, pdb id: 1rx2) is selected for its importance as a model

system widely used to explain the basis of antibiotic resistance. We investigate the origins of resistance invoking point mutations, particularly at positions (P21, D26, L28, W30, I94, F153) that are significant in previous experimental studies. In a statistical analysis over all the proteins in the test set, we find that residues with high  $\Delta L$  and  $\Delta BC$  have large conservation scores for each protein in the set. The former measure distinguishes positions that can steer large conformational changes while the latter differentiates sites that are responsible for communicating information between domains.

In fact, while  $\Delta L$  provides information on signaling endpoints,  $\Delta BC$ , is a measure of the residues controlling the signals. One may think of them as the essential nodes in the system describing a minimal network structure. All other interactions are redundant connections overlaid on this essential network, ensuring proper functioning under the many perturbations received from the environment.

**Monday 5 September****9:00–11:00, Hall D****Mechanisms of pro-inflammatory diseases****S-04.02.2-001****A variety of cancer-associated inflammatory reactions**

**Y. Ben-Neriah<sup>1</sup>, A. Lasrey<sup>1</sup>, D. Aran<sup>2</sup>, A. Zinger<sup>1</sup>, S. Finkin<sup>1</sup>, I. Stein<sup>1</sup>, D. Yuan<sup>3</sup>, A. Hellman<sup>4</sup>, M. Heikenwalder<sup>3</sup>, E. Pikarsky<sup>1</sup>**  
<sup>1</sup>Lautenberg Center for Immunology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel, <sup>2</sup>Institute for Computational Health Sciences, University of California, San Francisco, United States of America, <sup>3</sup>Institute of Virology, Technische Universität München, Helmholtz Zentrum München, Munich, Germany, <sup>4</sup>Developmental Biology and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Inflammation has many faces, most commonly observed as an acute reaction in response to pathogen or another insult, or a chronic disease, accompanying persistent infection and excessive immune activation, such as hepatitis and inflammatory bowel disease. Whereas these two and a few other examples of chronic inflammation have been widely recognized as drivers for a minority of cancer types, the role of inflammation in most cancers is still elusive. We have recently characterized certain non-traditional forms of inflammation, among which are focal inflammation and parainflammation, an unfamiliar type of smoldering inflammation, which appear to affect many cancer diseases. We showed that liver inflammation foci bearing features of ectopic lymphoid structures nurture liver cancer progenitors and are associated with bad prognosis of liver cancer and will discuss their role in hepatocellular carcinoma in the context of cancer immunotherapy. Parainflammation (PI) is a low grade inflammatory process, triggered by intrinsic cellular stressors, such as persistent DNA damage response, which cannot be observed even microscopically, and is detected only by molecular analysis (gene and protein expression). Using mouse models, we demonstrated that PI is associated with cellular senescence and cooperates with the tumor suppressor effect of p53, yet in the absence or mutation of p53, converts from a growth inhibitory to growth and cancer promoting mechanism. We detected PI in a variety of human tumors, nearly 30% of all human cancer, and will discuss the impact of parainflammation in cancer progression and means of suppressing it for preventing cancer.

**S-04.02.2-002****p62/SQSTM1-a key mediator of stress-induced liver cancer**

M. Karin

*UC San Diego, La Jolla, United States of America*

p62/SQSTM1 is an autophagy adaptor as well as a signaling platform involved in activation of several signaling pathways including the NRF2 antioxidant defense, the NF- $\kappa$ B mediated inflammatory response and mTORC1-dependent nutrient sensing. Although expressed in many cell types including macrophages, p62 plays a particularly important role in liver pathophysiology. First and foremost, p62 is a major component of Mallory-Denk bodies and hyaline granules, inclusion bodies that are prominent pathological features of degenerative liver diseases and hepatocellular carcinoma (HCC), respectively. The mechanisms that lead to formation of p62 containing inclusion bodies are not entirely clear, but they likely involve increased p62 synthesis, which is regulated at the transcriptional level by NRF2 and NF- $\kappa$ B and post-transcriptionally through decreased p62 degradation, due to attenuation of its autophagic clearance. In addition to its role in promoting the autophagic-lysosomal degradation of damaged proteins and organelles, p62 is needed for maintenance or NRF2 which is responsible for inhibition of oxidative stress oxidative stress and toxic liver damage. However, chronic elevation of p62 expression contributes to HCC development by preventing oncogene induced senescence and death of cancer initiating cells.

**S-04.02.2-003****RNA regulators of the inflammatory response**

S. Ghosh

*College of Physicians & Surgeons, Columbia University, New York, United States of America*

Sepsis, a form of systemic inflammation that results from bloodstream infection, is increasing in prevalence and continues to have a 28–50% mortality rate, suggesting new immunomodulatory treatments are needed. Although injection of high doses of the bacterial cell wall component lipopolysaccharide (LPS) can mimic septic shock, prolonged exposure to sub-lethal doses induces a state of LPS tolerance. LPS tolerance has two main effects: the attenuation of subsequent inflammatory responses, and the enhancement of microbial clearance. This has made artificial induction of LPS tolerance an attractive goal for sepsis treatment. However, the molecular mechanisms underlying LPS tolerance remain poorly understood. This has undermined efforts to create drugs and animal models to safely induce tolerance and ascertain whether it is truly of clinical benefit in sepsis treatment. To provide insight into tolerogenic mechanisms, we sought to identify novel microRNA (miRNA) mediators of tolerance. By screening for tolerance-associated miRNAs in an *in vitro* macrophage model, we identified a few miRNAs as a potential regulators of prolonged inflammatory responses. Upregulation of these miRNAs selectively suppresses the expression of a specific subset of inflammatory genes, while leaving expression of other regulatory and antimicrobial genes intact. This effect is mediated through direct targeting of chromatin-modifying complexes. Hence, miRNAs may coordinate and reinforce the chromatin modification and gene expression changes required for LPS tolerance. This work may reveal new targets for sepsis drug development and provide new models to test tolerance function and therapeutics *in vivo*.

**ML-04.02.2-001****Nutritional modification of endoplasmic reticulum stress and inflammasome activity by a bioactive lipokine prevents atherosclerosis**B. Kocaturk<sup>1</sup>, I. Çimen<sup>1</sup>, Ö. Tufanlı<sup>1</sup>, S. Koyuncu<sup>1</sup>, O. Apaydin<sup>1</sup>, U.I. Onat<sup>1</sup>, G.S. Hotamisligil<sup>2</sup>, E. Erbay<sup>1</sup><sup>1</sup>*Molecular Biology and Genetics Department, Bilkent University Ankara, Turkey,* <sup>2</sup>*Harvard T.H Chan School of Public Health, Boston, United States of America*

Atherosclerosis is the leading cause of death in the civilized world. The disease progresses as macrophages engulf lipids and accumulate in the vessel wall. The newly formed foam cells release pro-inflammatory cytokines which is crucial in disease pathogenesis. It was previously shown that the level of saturated fatty acids (SFAs) is elevated in the plasma of patients with atherosclerosis. Interestingly, SFAs do not only trigger Endoplasmic Reticulum (ER) stress but also activate inflammasome, of which both have pro-atherogenic role. Based on the aforementioned findings, we hypothesize that in contrast to SFAs, unsaturated fatty acids, in particular Palmitoleate (PAO), has a remedial effect on plaque formation. Our studies show that systemic PAO treatment evokes an overall lipidomic remodeling in mice that is associated with resistance to lipid-induced ER stress, production of mitochondrial reactive oxygen species and inflammasome activation in macrophages. In addition, PAO uptake decreases pro-inflammatory IL-1 $\beta$  levels in serum and atherosclerotic plaque, reduces ER stress in plaque resident macrophages and plaque size in a mice model of atherosclerosis. These findings demonstrate that external supplementation of a product of de novo lipogenesis such as PAO can promote metabolic resilience of organelles and limit the progression of atherosclerosis. Overall, these findings indicate that PAO supplementation could be a desirable therapeutic approach for metabolic and inflammatory diseases.

**ML-04.02.2-002****Dickkopf-1 and interleukin 6 interaction as a potential mechanism of Wnt pathway inhibition in rheumatoid arthritis**K. Malysheva<sup>1,2</sup>, K. de Rooij<sup>3</sup>, C. W. G. M. Löwik<sup>3</sup>, D. L. Baeten<sup>4</sup>, S. Rose-John<sup>5</sup>, R. Stoika<sup>1</sup>, O. Korchynskyi<sup>1,3,4</sup><sup>1</sup>*Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv, Ukraine,* <sup>2</sup>*Laboratory of Molecular Biology and Clinical Biochemistry, Institute of Animal Biology of National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine,* <sup>3</sup>*Leiden University Medical Center, Leiden, the Netherlands,* <sup>4</sup>*Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, the Netherlands,* <sup>5</sup>*Institute of Biochemistry, Christian-Albrechts-University, Kiel, Germany*

**Introduction:** Rheumatoid arthritis (RA) is a severe autoimmune inflammatory disorder, which etiology remains poorly understood. It has been demonstrated that tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin (IL) 6 play a crucial role in RA pathophysiology. Skeletal homeostasis is hampered by the TNF-mediated expression of Dickkopf-1 (DKK-1), which suppresses Wnt signals that are critical for joint maintenance and remodeling.

**Aim:** To investigate in detail the interaction between DKK-1 and IL6 as a potential mechanism of Wnt pathway inhibition in RA.

**Materials and Methods:** We used luciferase reporter assays to monitor Wnt pathway activation upon IL-6, TNF $\alpha$  and DKK-1 treatment. *In vitro* evaluation of functional contribution of IL-6 and TNF $\alpha$  interaction to inhibition of bone formation was

performed by using lentiviral small hairpin RNAs (shRNA) knockdown in mouse mesenchymal precursor cells (MPC) of C2C12 and KS483 lines induced to differentiate into osteoblasts by BMPs.

**Results:** In our preliminary studies we described a previously unrecognized negative interaction between the Wnt and IL-6 signaling pathways in skeletal tissues as a potential major mechanism leading to age- and inflammation-related destruction of bone and joints. We found that IL-6 inhibits activation of Wnt signaling in primary human synoviocytes, and IL-6 cooperatively with TNF $\alpha$  and DKK-1 inhibits the activation of Wnt response. ShRNA-mediated knockdown of *IL-6* mRNA significantly increased early BMP2/7-induced osteogenesis and rescued it from the negative effect of TNF $\alpha$  in C2C12 cells as well as intensified bone matrix mineralization in KS483 cells.

**Discussion and conclusion:** IL-6 cooperates with TNF $\alpha$  and with DKK-1 in inhibition of osteogenic Wnt signaling that is crucially important for joint maintenance and remodeling. Currently, direct protein-protein interactions between IL-6 and DKK-1 as a possible major mechanism mediating anti-osteoblastic effects of IL-6 on Wnt signaling are under our evaluation.

## Monday 5 September 9:00–11:00, Hall E

### Epigenetics and cancer

#### S-05.02.2-001

#### Mammalian heterochromatin in normal and perturbed development

T. Jenuwein

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Epigenetic mechanisms, such as histone modifications, DNA methylation, nucleosome remodeling and non-coding RNA control eukaryotic development beyond DNA-stored information. These machineries work together to organize chromatin into accessible (euchromatic) and inaccessible (heterochromatic) domains. Mammalian heterochromatin is characterized by its underlying repetitive DNA sequence and epigenetic hallmarks, such as H3K9me3 methylation, DNA methylation and non-coding repeat RNA. Despite the identification of these key components, it is still unclear how heterochromatin is initiated and which mechanisms maintain heterochromatin structure. I will discuss novel insights into how repeat-rich DNA and RNA interact with chromatin-modifying enzymes and nucleosomes to enforce mammalian heterochromatin formation. Since heterochromatin has important functions in safeguarding genome integrity, in silencing of endogenous retroviruses, in preserving cell type identities and in stabilizing gene expression programs, a better understanding of the establishment and maintenance of mammalian heterochromatin will yield new insights into the plasticity of cell fate decisions and provide novel strategies to modulate epigenetic control in normal and perturbed development.

#### S-05.02.2-003

#### DNMT and HDAC inhibitors globally induce cryptic TSSs encoded in long terminal repeats

D. Brocks, C. R. Schmidt, M. Daskalakis, D. Li, J. Li, H. S. Jang, B. Zhang, D. B. Lipka, J. Schott, H. Bierhoff, Y. Assenov, M. Helf, A. Ressenrova, A. Lindroth, S. Haas, M. Essers, C. D. Imbusch, B. Brors, I. Oehme, O. Witt, M. Lübbert, G. Stoecklin, C. Gerhäuser, C. C. Oakes, T. Wang, C. Plass

*DKFZ, Heidelberg, Germany*

Several mechanisms of action have been proposed for DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi); mainly based on candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences. By mapping global transcription start site (TSS) and chromatin dynamics, we observed the activation of thousands of cryptic, currently non-annotated TSSs (TINATS) following DNMTi and/or HDACi treatment. The resulting transcripts encode truncated or chimeric open reading frames that can be translated into products with predicted abnormal functions or immunogenic potential. TINAT activation after DNMTi coincided with DNA hypomethylation and gain in H3K4me3, H3K9ac, and H3K27ac histone marks. In contrast, HDACi induced only canonical TSSs in association with histone acetylation, but TINATs via a yet unknown mechanism. Nevertheless, both inhibitors convergently induced unidirectional transcription from identical sites since TINATs are encoded in solitary long-terminal repeats of the endogenous retrovirus-9 family, epigenetically repressed in virtually all normal cells.

#### S-05.02.2-002

#### The epigenetics of prostate cancer

R. Santoro

*University of Zurich, Zurich, Switzerland*

Prostate cancer (PCa) is one of the most common types of cancer in men. PCa has an unpredictable clinical history: while most tumors are indolent, some patients display lethal phenotypes. Treatment options for metastatic disease are not curative since patients inevitably acquire resistance and relapse. A particular feature of PCa is the lack of somatic mutations and an altered epigenome. We have recently shown that elevated expression of the epigenetic factor TIP5 in tumors is tightly associated with a molecular subtype displaying a CpG island methylator phenotype (CIMP) and serves as an independent predictor of biochemical recurrence. TIP5 establishes epigenetic silencing of genes commonly repressed in metastatic tumors, implicated in developmental processes and required for proliferation, invasion and stem-like features. PCa is of epithelial nature and cancer stem cells (CSCs) are considered an enticing explanation for relapse, metastasis and therapy failure and thus an important target for effective therapy for advanced PCa. To determine whether TIP5 might represent a therapeutic target for metastatic PCa, we have established culture conditions to isolate cells with stem properties (stem-like PCa cells) from the heterogeneous PC3 metastatic PCa cell line. RNAseq analysis revealed that obtained stem-like PCa cells resemble molecular stem features such as downregulation of developmental and differentiation genes which are often repressed in metastatic tumors and upregulation of CSC markers such as *NESTIN* and *ALDH1A2*. Remarkably, TIP5 is required for the formation of stem-like PCa cells, indicating TIP5 as a potential therapeutic target in PCa stem cells. Data will be shown concerning the role of TIP5 in PCa stem-features and strategies to specifically inactivate TIP5 function in PCa.



**ML-05.02.2-002****Temporal and spatial epigenome editing allows precise gene regulation**

M. Adli

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Ability to precisely control the expression a gene in the human genome has great implications for basic and clinical research. Here, we develop and utilize CRISPR based epigenetic tools and alternative targeting approaches to control the temporal duration as well as the intensity of gene expression through epigenome editing. To control temporal gene expression and study the induced epigenetic memory, we integrated locus specific epigenome editing tool with plant based auxin-induced degradation (AID) system. To control the amplitude of gene expression from a specifically targeted endogenous locus, we used dCas9-P300 epigenetic editing tool to reprogram non-regulatory distal genomic regions into enhancer-like elements. We show that by controlling the target site distance to the promoter region, the gene expression intensity can be precisely regulated through such "induced enhancer" (IE) sites. These approaches allow novel insight into gene regulation from distal regulatory sites and epigenetic memory induced by specific epigenetic marks

**Monday 5 September****15:30–17:30, Hall A****Novel signaling pathways controlling the cardiac function****S-06.01.2-002****Signaling pathways involved in cardiac energy metabolism**

J. F. Glatz, D. Neumann, J. J. Luiken

*Maastricht University, Maastricht, the Netherlands*

The heart is a continuously active biological pump that converts chemical energy into mechanical energy. Various signaling pathways are of the utmost importance to coordinate energy metabolism and contractile function and to allow the heart to respond to changes in demand. This lecture will focus on the significance of these signaling pathways in both the healthy and diseased heart and in particular address the pathways involved in the uptake and utilization of the predominant substrates for energy production, long-chain fatty acids and glucose.

A decrease in cardiac energy (ATP) content induces activation of AMP-activated protein kinase (AMPK). This cellular energy sensor then positively regulates signaling pathways that replenish cellular ATP supplies while negatively regulating ATP-consuming biosynthetic processes. The AMPK-activated signaling events orchestrate the simultaneous upregulation of substrate uptake (by translocating the fatty acid transporter CD36 and the glucose transporter GLUT4 from endosomes to the sarcolemma) and substrate oxidation (by activating the enzymes involved in both pathways). Recent studies have shown that contraction-induced GLUT4 translocation also requires the activation of protein kinase D1 (PKD1). Such dual input necessity (AMPK and PKD1) might serve to limit increases in cardiac glucose uptake to specific circumstances.

Insulin also stimulates both cardiac CD36-mediated fatty acid and GLUT4-mediated glucose uptake but, in contrast to AMPK, activates anabolic processes (glycogen and triacylglycerol synthesis). The insulin and AMPK signaling cascades are complex and

their cross-talk is only beginning to be understood. Interestingly, in the insulin-resistant and diabetic heart, glucose uptake and utilization is impaired while fatty acid uptake and utilization is markedly increased. AMPK activation has been suggested as therapeutic approach for the diabetic heart.

**S-06.01.2-001****Signaling mechanisms regulating cardiac muscle growth and atrophy**

S. Schiaffino

*Venetian Institute of Molecular medicine (VIMM), Padova, Italy*

The dissection of the signaling pathways that control cardiac muscle growth and atrophy is essential to understand the adaptive changes induced by altered heart function, such as overloading and unloading, and to take appropriate countermeasures to control the development of heart failure. In this presentation, I will review studies on skeletal muscle, showing the role for various pathways acting as positive or negative regulator of skeletal muscle growth, including myostatin, IGF-1, calcineurin-NFAT and MEF2, then discuss their relevance in cardiac muscle. A parallel comparison will be proposed for the mechanisms responsible for muscle atrophy, focusing on the ubiquitin-proteasomal and autophagic-lysosomal systems.

**ML-06.01.2-001****A novel mechanism to explain glucose uptake inhibition by fatty acids**Y. Angin<sup>1</sup>, E. Renguet<sup>1</sup>, P. Morue<sup>1</sup>, A. Ginion<sup>1</sup>, C. Beauloye<sup>1</sup>, S. Horman<sup>1</sup>, L. Bertrand<sup>1</sup>*<sup>1</sup>Université catholique de Louvain, Institut de Recherche Expérimentale et Clinique (IREC), Pole of Cardiovascular Research, Brussels, Belgium*

Continuous contractile functions of heart requires high energy demand which is met by the oxidation of carbon sources substrates such as fatty acids, glucose, ketone bodies, lactate etc. Under physiological conditions the heart can switch between available substrates to ensure a continuous ATP production. However under conditions of increased circulating fatty acids such as during fasting or diabetes, heart switches its energy production towards the use of fatty acids, which is accompanied by the decreases of insulin-mediated glucose uptake into heart. Both literature and previously obtained data in the lab allow us to hypothesize that protein acetylation process can contribute to the molecular mechanisms involved in glucose uptake suppression by the acute and chronic oxidation of alternative substrates including fatty acids, oxidation of which give rise to Acetyl-CoA formation. Primary adult rat cardiomyocytes were incubated with oleate and palmitate and insulin-stimulated glucose uptake, insulin signaling, translocation of GLUT4, total lysine acetylations were measured. We showed that insulin-stimulated glucose uptake is inhibited dose and time dependently upon fatty acid exposures without changes in insulin signaling. These findings are consistent with the diminished plasma membrane GLUT4 levels and increased total lysine acetylation. Interestingly, pharmacological decrease in global protein acetylation increased basal and insulin-stimulated glucose uptake. These findings suggest that protein acetylation processes are involved in inhibition of glucose uptake upon fatty acids treatment targeting GLUT4 translocation to plasma membrane. Findings of this project will not only help the unknowns of Randle effect but also will provide new putative targets to treat/prevent diseases accompanied with impaired metabolic flexibility.

**Monday 5 September**  
**15:30–17:30, Hall B**

**Mechanism of neurodegenerative diseases**

**S-09.02.2-002**

**Biology of mammalian prions**

**A. Aguzzi**

*Institute of Neuropathology University Hospital Zurich, Zurich, Switzerland*

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of humans and many animal species caused by prions. The main constituent of prions is PrP<sup>Sc</sup>, an aggregated moiety of the host-derived membrane glycolipoprotein PrP<sup>C</sup>. Prions were found to encipher many phenotypic, genetically stable TSE variants. The latter is very surprising, since PrP<sup>C</sup> is encoded by the host genome and all prion strains share the same amino acid sequence. Here I will review what is known about the infectivity, the neurotoxicity, and the neuroinvasiveness of prions. Also, I will explain why I regard the prion strain question as a fascinating challenge – with implications that go well beyond prion science. Finally, I will report some recent results obtained in my laboratory, which is attempting to address the strain question and some other basic issues of prion biology with a “systems” approach that utilizes organic chemistry, photophysics, proteomics, and mouse transgenesis.

**S-09.02.2-003**

**The intertwined roles of RNA misregulation and protein aggregation in ALS and FTD**

**M. Polymenidou**

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The RNA-binding protein TDP-43 aggregates and mislocalizes in the vast majority of amyotrophic lateral sclerosis (ALS), as well as approximately half of frontotemporal dementia (FTD) cases. Mutations in TDP-43 and other genes lead to familial ALS, but the majority of cases are sporadic (sALS) with no known genetic errors. Cytoplasmic TDP-43 aggregates correlate with neurodegeneration, indicating their key neurotoxic role, which may be mediated via sequestration of target RNAs and interacting proteins. While the mechanism of pathological TDP-43 aggregation remains unknown, its incorporation in physiological, cytoplasmic stress granules via its highly aggregation-prone, low complexity domain have been hypothesized to initiate aggregation, albeit no definite experimental demonstration of such a physiopathological transition exists to date.

To understand the biochemical properties and exact protein and RNA composition of pathological TDP-43 aggregates, we combine complex biochemical analysis with unbiased mass spectrometry and RNA sequencing of human samples. We show that TDP-43 forms a major pathological population of large and buoyant assemblies in sALS, which contain several proteins, including a significant representation of RNA-binding proteins with low complexity domains. To initiate the pathological TDP-43 complex formation in order to study the molecular events triggered by aggregation of these ALS/FTD-linked proteins, we use preformed TDP-43 oligomers on mouse organotypic hippocortical and spinal cord slice cultures. Preformed oligomers of TDP-43 were rapidly internalized by microglia and neurons and caused its endogenous isoform to oligomerize in the cytoplasm, accompanied by pathologic posttranslational modifications, such as phosphorylation of TDP-43, which intensified and matured into compact, ubiquitinated structures over time in culture.

**S-09.02.2-001**

**The calcium channel subunit Alpha2delta2 suppresses axon regeneration in the adult CNS**

**F. Bradke**

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Injuries to the adult central nervous system (CNS) often result in permanent disabilities because neurons lose the ability to regenerate their axon during development.

Here, whole transcriptome sequencing and bioinformatics analysis followed by gain- and loss-of-function experiments identified *Cacna2d2*, the gene encoding the Alpha2delta2 subunit of voltage gated calcium channels (VGCCs), as a developmental switch that limits axon growth and regeneration.

*Cacna2d2* gene deletion or silencing promoted axon growth *in vitro*. *In vivo*, Alpha2delta2 pharmacological blockade through Pregabalin (PGB) administration enhanced axon regeneration in adult mice after spinal cord injury (SCI).

Gabapentinoids such as gabapentin and PGB also improved functional recovery in mice and neurological function in SCI patients. Thus, our findings suggest that targeting Alpha2delta2 may be a novel treatment strategy to promote structural plasticity and regeneration following human CNS trauma.

**ML-09.02.2-001**

**Microtubule-associated protein 1B is dysregulated in spinal muscular atrophy**

**G. Bora-Tatar<sup>1,2</sup>, S. Rademacher<sup>2,3</sup>, N. Hensel<sup>2</sup>, P. Claus<sup>2,3</sup>, H. Erdem-Yurter<sup>1</sup>**

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Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease, characterized by symmetrical muscle weakness and atrophy. SMA is primarily caused by homozygous loss of the *Survival of Motor Neuron 1* (SMN1) gene, which results in reduced amount of SMN protein. Although SMN is ubiquitously expressed in all cell types, lower motor neurons preferentially degenerate upon SMN protein reduction. Previously, we and others reported SMN-dependent alterations of proteins which regulate the neuronal cytoskeleton. Recent studies also showed differential regulation of microtubule modulating proteins in SMA models. Microtubule-associated protein 1B (MAP1B) is a cross-linker protein that interacts with both actin filaments and microtubules and plays a role in microtubule dynamics, an essential process for proper neuronal morphology and function. In this study, we further investigated the contribution of MAP1B protein in SMA pathomechanism.

We investigated total and phosphorylated MAP1B protein levels in different SMA model systems. Spinal cords of severe SMA mice at different postnatal stages showed a significant increase in total MAP1B protein levels in pre-symptomatic mice and motoneuron-like NSC34 cells with a SMN knock-down. Additionally, symptomatic mice displayed a MAP1B hyperphosphorylation at a phospho-site which is a known target of GSK3 $\beta$ -kinase. Accordingly, inhibitory phosphorylation of GSK3 $\beta$ -kinase remained unchanged.

Our results suggest that MAP1B protein is dysregulated in SMA models, both *in vitro* and *in vivo*. However, we will investigate MAP1B alterations in an ongoing study in patients. Finally,

the functional consequences of MAP1B dysregulation will increase our understanding about modulation of the cytoskeleton in SMA.

### ML-09.02.2-002 hGPR17 signaling cascade effects mitochondrial function

M. Kandhavelu

*Tampere University of Technology, Tampere, Finland*

G-protein coupled receptor 17, GPR17, signaling has been linked to several diseases including Multiple Sclerosis, Parkinson's and Alzheimer's. GPR17 is a P2Y-like receptor expressed majorly in the brain, heart, kidney and umbilical vein of endothelial cells. Upon activation, GPR17 triggers the binding of secondary messenger inositol 1,4,5-trisphosphate (InsP3) to the InsP3 receptors on the endoplasmic reticulum, which in turn leads to the release of Ca<sup>2+</sup> ions. Previous reports have suggested that changes in Ca<sup>2+</sup> level alter the mitochondrial Ca<sup>2+</sup>, mitochondrial functioning, and cell survival. However, the signaling link between GPR17 and mitochondria is poorly understood. Here, we assess the previously unknown effects of GPR17 signaling on mitochondrial function using single cell imaging, molecular signal processing, and high throughput screening. For that, we first measured mtROS and Ca<sup>2+</sup> levels in single cells upon activation of hGPR17 using known agonist and antagonist in 1321N1 cells transfected with the hGPR17 gene. Also, we tested novel small molecules that were computationally predicted to be potential activators of hGPR17. Next, we blocked mitochondrial functioning using electron transport chain inhibitors and observed the changes in hGPR17 cascade mediated mtROS and Ca<sup>2+</sup> levels. Our findings suggest a broader role for hGPR17 on mitochondria and hence can be exploited as a potential therapeutic agent for regulating mitochondria-related diseases.

**Tuesday 6 September**  
**9:00–11:00, Hall A**

### MicroRNAs and noncoding RNAs

#### S-01.03.3-002 RNA binding proteins as modulators of coding and non-coding RNA pathways

G. Meister

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In animals, microRNAs (miRNAs) are transcribed as capped and polyadenylated primary transcripts. Mature miRNAs are processed from these transcripts by the subsequent action of the two RNase III enzymes Droscha and Dicer. In the cytoplasm, miRNAs directly bind to a member of the Argonaute (Ago) protein family and guide it to partially complementary target sites on mRNAs leading to inhibition of gene expression. In addition to the biogenesis pathways described above, non-canonical miRNA biogenesis have been found. MiRNAs can be independent of Droscha or Dicer but nevertheless are found in functional gene silencing complexes. Here, we report that RNA-binding proteins can be involved in non-canonical miRNA pathways.

The Lupus autoantigen La is an RNA-binding protein that stabilizes RNA polymerase III (pol III) transcripts and supports RNA folding and has in addition been implicated in the mammalian microRNA (miRNA) pathway. We have analyzed effects of La depletion on Argonaute (Ago)-bound small RNAs in human cells. We find that in the absence of La, distinct tRNA

fragments are loaded into Ago proteins. Thus, La functions as gatekeeper ensuring correct tRNA maturation and protecting the miRNA pathway from potentially functional tRNA fragments. However, one specific isoleucine pre-tRNA produces both a functional tRNA and a miRNA even when La is present. We demonstrate that the fully complementary 5' leader and 3' trailer of the pre-tRNA-Ile form a double stranded RNA molecule that has low affinity to La. Instead, Exportin-5 (Xpo5) recognizes it as miRNA precursor and transports it into the cytoplasm for Dicer-processing and Ago-loading.

#### S-01.03.3-003 Mechanisms governing amplification of short interfering RNAs by RNA dependent RNA polymerase

P. Brodersen

*Department of Biology, University of Copenhagen, Copenhagen, Denmark*

Small RNA guided gene silencing may involve positive feedback loops in which an RNA species targeted by a primary small RNA bound to an ARGONAUTE (AGO) protein leads to production of new amplified populations of small RNAs directed against the same target. Such positive feedback loops confer robust, and potentially irreversible, silencing, and are often at play in plant silencing of viral and transgene RNA. The central factor of small RNA amplification is the enzyme RNA dependent RNA Polymerase that synthesizes doublestranded RNA using as template RNA species targeted by a primary small RNA. Most endogenous transcripts do not instigate establishment of amplification loops via RNA dependent RNA Polymerase, but may do so upon mutation of RNA processing or decay pathways. These observations have supported a view in which aberrant RNAs are triggers of RNA dependent RNA Polymerase activity. We will discuss data that support the alternative view that RNA dependent RNA Polymerase is recruited directly by AGO proteins, and that the dwell time of AGO on target RNA may be crucial to determine whether efficient recruitment of RNA dependent RNA Polymerase occurs or not.

#### S-01.03.3-001 Another front in the miRNA research: alternative polyadenylation

A. Erson Bensan

*METU, Ankara, Turkey*

mRNA 3' UTRs have long been known to have important roles in maintaining the stability, localization and half-life of the mRNAs. Discovery of microRNAs and RNA-binding proteins (RBPs) contributed to the understanding of how 3'UTRs regulate gene expression in normal and in disease states. In addition, advancements in sequencing and transcriptome analysis methods revealed that majority of human genes harbor multiple polyadenylation signals on their 3' UTRs, that can be differentially selected based on the physiological state of cells, resulting in alternative 3' UTR isoforms. Therefore, alternative polyadenylation (APA) has been attracting attention due to roles in gene expression regulation generally by shortening of 3'UTRs upon proliferative signals. Consequently, APA generated isoforms may have shortened 3' UTRs to provide an escape route from the repressive effects of microRNAs and/or RBPs. We are interested in identifying and characterizing deregulated APA events in breast cancers combining computational and experimental tools. Detection of 3'UTR length alterations of various genes may help the discovery of new cancer related genes, which may have been overlooked in conventional gene expression analyses. Overall,

detection of such isoforms in cancer may implicate some proto-oncogene activation cases of unknown causes and contribute to a better understanding of molecular mechanisms of cancer.

Funding for our APA work is provided by TUBITAK 112S478 and 114Z884.

### ML-01.03.3-001

#### Can we trust miRNA-mRNA prediction tools?

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miRNAs are small RNAs, which are formed global network in regulation of gene expression at the post-transcriptional level. There is no any genes, which couldn't regulated by miRNAs. However, the question about the identification of miRNA-mRNA interaction is still open. There are five widely used predictive tools (TargetScan, Pictar2, PITA, RNA22 and miRanda). They have different approaches to make prediction. It was impossible to understand, which one is better and can we trust these predictions. However recently the new high-throughput experimental method «CLASH» was developed to identify all miRNA-mRNA interactions. It allows to find out all miRNA-mRNA cases in HEK293 cell line.

Using python 3.5.1, we created an algorithm to analyse and compare data from «CLASH» experiment with predicted miRNA sites by all five algorithm. Expression data for mRNA and miRNA were used from FANTOM5 and GEO DataSets.

For estimating efficiency of miRNA-mRNA prediction tools, we used next parameters: sensitivity, positive predicted value, predictions in different mRNA regions (3'UTR, CDS, 5'UTR), predictions for different types of interactions (5 classes), predictions of «canonical» and «nocanonical» interactions. Also we made the same analyze using random data for miRNA binding sites. We analyzed variety of miRNAs – 386 different miRNAs was identified in «CLASH» data and 384 – in prediction data. But the overlapping is only 44% (168 miRNAs). Expression analyze of miRNA revealed several interesting groups: highly expressed miRNAs without any interactions, highly expressed miRNAs with small number of interactions and lowly expressed miRNAs, which take a part in a lot of interactions with mRNAs.

Big discrepancy between experimental data and prediction, lead us to conclusion, that all softwares couldn't make positive prediction, and more importantly – any overlapping of softwares couldn't insure sufficient good result.

### ML-01.03.3-002

#### MicroRNA-related gene polymorphisms and idiopathic azoospermia

M. Balkan<sup>1</sup>, O. I. Ay<sup>2</sup>, M. E. Erdal<sup>2</sup>, A. Rustemoglu<sup>3</sup>, M. Atar<sup>4</sup>, N. K. Hatipoglu<sup>4</sup>, I. Yildiz<sup>5</sup>, H. Akbas<sup>6</sup>, Ü. Karakas<sup>2</sup>, M. N. Bodakçi<sup>4</sup>

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Small noncoding RNA molecules, such as microRNAs, have a role in the diversity of cellular functions. Although it is known

that global suppression of miRNA biogenesis pathway genes leads to cancers, the effects of common genetic variants of these genes on male infertility is unclear. To better understand this effect, in this case-control study we genotyped six single-nucleotide polymorphisms (SNPs) located on microRNA biogenesis pathway genes, using real-time polymerase chain reaction. A total of 108 infertile men with idiopathic azoospermia and 125 fertile control subjects were included in the study, and the associations between individual and combined genotypes and idiopathic azoospermia were analyzed. The individual AA genotype frequency of the *GEMIN3* (rs197388) gene was found to differ significantly between the patient and control groups; thus, the AA genotype may be regarded as indicative of a higher predisposition for idiopathic azoospermia. The combined genotype analysis, including six SNPs, revealed statistically significant differences for some combinations between the patients and control subjects. For example, the AA\CA-CC-TT-AT genotype combination for the *XPO5-RAN-DICER1-GEMIN3* combined loci was significantly different, and it may have a predisposition for idiopathic azoospermia. According to our results, both individual and combined genotypes of SNPs from miRNA genes may be used to predict the risk of male infertility with idiopathic azoospermia.

**Tuesday 6 September**  
**9:00–11:00, Hall B**

### Autophagy: Regulation mechanisms

#### S-02.03.3-001

#### Novel ATG5 interactors in the control of basic autophagy and mitophagy

D. Gozuacik

Sabanci University, Istanbul, Turkey

In our lab in Sabanci University, Istanbul, we focus on signaling events regulating mammalian autophagy in health and disease. To discover new autophagy regulators and coordinators, we performed several unbiased functional screens.

Our microRNA (miRNA) screens led to the discovery of several miRNAs targeting autophagy at various steps of the pathway. miRNAs are able to affect the expression of a number of proteins at once. Therefore, miRNA networks seem to integrate cellular stress response pathways including autophagy and apoptosis, and coordinate them to shape cell faith. Our published and unpublished results allowed us to have a better picture of the miRNA networks modulating autophagic responses in human health and disease.

Protein interaction screens performed in our lab led us to discover novel proteins involved in autophagy regulation. In fact, some of these proteins were directly interacting with the core autophagy machinery components. Unexpected direct links between autophagy and other important cellular pathways were found, allowing us to reveal novel entry points for autophagy regulation and coordination in cells. Interestingly, some of this interactions seemed to be autophagy signal specific, and our work revealed novel dynamics in autophagy regulation.

Results from our recently published and unpublished studies will be presented and implications of our results in human health problems, including cancer and degenerative diseases, will be discussed.

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**ML-02.03.3-001****Activation of C/EBPbeta-3 during cellular differentiation links the development of ER stress and autophagy in colon epithelial cells**A. Sade Memisoglu<sup>1,2</sup>, S. Tuncer<sup>1</sup>, S. Banerjee<sup>1</sup><sup>1</sup>Middle East Technical University, Ankara, Turkey, <sup>2</sup>Dokuz Eylul University, Ankara, Turkey

The molecular mechanisms of balanced and continuous generation of intestinal epithelial cells, is closely regulated and disruption of this balance may result in neoplastic transformation and malignant growth. Differentiation is regulated by numerous signals, which in turn regulate signaling pathways directing activation or inactivation of certain transcription factors. Perturbations like changes in Ca<sup>2+</sup> levels, glucose or amino acid starvation may result in an ER stress response, which is also implicated in the differentiation process. In addition, ER stress and autophagy pathways may function together under certain circumstances. The transcription factor C/EBPbeta is implicated in differentiation, ER stress and autophagy.

In the current study, we have used Caco-2 and HT-29, two colorectal cancer cell lines that can undergo spontaneous differentiation after reaching 100% confluency, or through glucose deprivation respectively to enterocyte like cells. We have observed an ER stress response to be activated during the process of differentiation. Ca<sup>2+</sup> flux into the cytoplasm was found to be the mediator of ER stress response in Caco-2 cells. Interestingly, ER stress also caused induction of autophagy during differentiation in both cell lines. Moreover, ER stress resulted in the upregulation of C/EBPbeta-3, a short isoform of C/EBPbeta generated through alternative translation, which then orchestrated the activation of autophagy. ChIP-seq revealed that C/EBPbeta was enriched in the promoters of genes related to metabolism, junctional and membrane proteins, endocytosis and differentiation and proliferation in intestinal epithelial cells.

We have shown here for the first time that ER stress induced autophagy and the involvement of C/EBPbeta-3 in these processes in the differentiation of Caco-2 and HT-29 cell lines. These results suggest new regulatory mechanisms that may be of significance in the process of intestinal epithelial differentiation.

**ML-02.03.3-002****The role of PNPLA1 protein in lipophagy mediated regulation of lipid droplets**G. Önal<sup>1</sup>, Ö. Oral<sup>2</sup>, E.Z. Taskiran<sup>3</sup>, A. Yüzbasıoğlu<sup>4</sup>, A. Karaduman<sup>5</sup>, D. Gözüaçık<sup>6</sup>, S. Dökmeçi<sup>1</sup><sup>1</sup>Department of Medical Biology, Hacettepe University, Ankara, Turkey, <sup>2</sup>Sabancı University Nanotechnology Research and Application Center, Istanbul, Turkey, <sup>3</sup>Department of Medical Genetics, Hacettepe University, Ankara, Turkey, <sup>4</sup>Center for Biobanking and Genomics, Hacettepe University, Ankara, Turkey, <sup>5</sup>Department of Dermatology, Hacettepe University, Ankara, Turkey, <sup>6</sup>Sabancı University Faculty of Engineering and Natural Sciences Molecular Biology Genetics and Bioengineering Program, Istanbul, Turkey

Lipid droplets (LDs) that are neutral lipid storage depots regulate cellular lipid metabolism. Patatin-like phospholipase domain containing protein-1 (PNPLA1) which localizes on the surface of LDs is associated with Autosomal Recessive Congenital Ichthyosis (ARCI), which is one of hereditary keratinization disorders. Mutant PNPLA1 protein is thought to cause abnormal lipid accumulation by disturbing cellular membrane organization, membrane trafficking and endocytic pathways. The aim of this

study is to seek the effects of mutant PNPLA1 protein on lipophagy mediated degradation of LDs.

In this study, in fibroblast cell cultures isolated from ARCI patients with PNPLA1 mutations (p.Y245del, p.D172N) and control individuals, expression and localization analysis of PNPLA1 protein was determined by using immunostaining and immunoblotting techniques. In addition, LDs were fluorescently stained and their accumulation was quantitatively analyzed. In order to investigate lipophagy mediated degradation of LDs, immunofluorescent co-localization of LDs with autophagic and lysosomal markers (Atg5, LC3, LAMP1, Lysotracker<sup>®</sup>Red-DND-99) were detected and expression levels of certain markers (LC3, p62) were determined.

According to our results, expression of PNPLA1 protein in fibroblast cells was demonstrated for the first time and they were detected to be localized on the surface of LDs. In patient's fibroblast cells, abnormal accumulation and increase in size of LDs ( $P \leq 0.0001$ ) and absence of co-localization of autophagic markers with LDs were found. Also, protein expression levels of autophagic markers (LC3 and p62) between patient's and control cells were found to be significantly different ( $P \leq 0.01$ ).

As a result, abnormal lipid accumulation and defects in autophagic flux in patient cells indicate that PNPLA1 protein may have role in lipophagy mediated regulation of LDs. Advanced functional analysis of this protein might help to shed light on mechanisms related with disease pathology.

**Tuesday 6 September****9:00–11:00, Hall C****Structural biology: Membrane complexes and supercomplexes****S-03.03.3-003****Shedding light on structure and mechanism of respiratory complexes central to energy metabolism**C. Wirth<sup>1</sup>, W. Kao<sup>1</sup>, V. Zickermann<sup>2</sup>, U. Brandt<sup>3</sup>, C. Hunte<sup>1</sup><sup>1</sup>Institute for Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, BIOS Centre for Biological Signalling Studies, University of Freiburg, Freiburg, Germany, <sup>2</sup>Structural Bioenergetics Group, Institute of Biochemistry II, Medical School, Goethe-University, Frankfurt, Germany, <sup>3</sup>Nijmegen Center for Mitochondrial Disorders, Radboud University Medical Center, Nijmegen, the Netherlands

Life needs energy, which non-photosynthetic organisms or cells gain from breaking down energy-rich compounds and cellular respiration. Central for the process of energy conversion are the proton translocating respiratory complexes which generate an electrochemical proton gradient that fuels ATP synthesis. Dysfunction of these complexes has been linked to a number of hereditary and degenerative diseases and they constitute attractive drug targets. Here, X-ray structures of mitochondrial proton-pumping NADH:ubiquinone oxidoreductase (complex I) and ubiquinol:cytochrome c oxidoreductase (complex III) are reported, which provide insights in the mechanisms of the enzymes and reveal binding modes of inhibitory ligands and drugs. Both complexes release deleterious reactive oxygen species (ROS) as by-products of their reactions and the control of ROS production will be discussed.

**S-03.03.3-001****Mechanism of action of Tc toxins**

S. Raunser

*Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany*

Tripartite Tc toxin complexes perforate the host membrane by forming channels that translocate toxic enzymes into the host, including humans. The underlying mechanism is complex but poorly understood. In my talk I will present the high-resolution structure of a complete 1.7 MDa Tc toxin complex composed of TcA, TcB and TcC. TcA forms a long translocation pore that is surrounded by a shell domain including putative receptor-binding domains. TcB and TcC form a large cocoon, in which the toxic domain resides and is autoproteolytically cleaved. A high-resolution structure of TcA embedded in the membrane and functional studies enable us to explain the mechanism of membrane insertion of the toxin. Our results allow us to understand the mechanism of action of Tc toxins at molecular to atomic level and shed new light on the interaction of bacterial pathogens, such as the plague pathogen *Yersinia pestis*, with their hosts.

**S-03.03.3-002****Structural insights into the mechanisms of photosynthetic light energy conversion**

J. Shen

*Okayama University, Okayama, Japan*

Photosynthesis converts light energy into biologically useful chemical, and provides us with oxygen indispensable for aerobic life on the earth by the splitting of water molecules. The water-splitting and light energy conversion are carried out by Photosystem II (PSII) and photosystem I (PSI), two large membrane-protein complexes located in the thylakoid membranes of oxygenic photosynthetic organisms. We have solved the structures of both PSII and PSI, providing important clues to the mechanisms of water-splitting and energy transfer reactions in these supercomplexes. I will overview these structures and discuss their functional implications.

The structure of PSII has been analyzed at 1.9 Å resolution with synchrotron X-rays, which revealed the detailed organization of a Mn<sub>4</sub>CaO<sub>5</sub>-cluster, the catalytic center for water oxidation. Furthermore, in order to avoid radiation damage, we employed a femtosecond X-ray free electron laser (XFEL) to collect damage-free diffraction data, and analyzed the PSII structure at 1.95 Å resolution. These results revealed the detailed structure of the Mn<sub>5</sub>CaO<sub>5</sub>-cluster, and established the basis for elucidating the mechanism for water-splitting.

PSI is connected with light-harvesting complex I (LHCI) in higher plants, forming a PSI-LHCI supercomplex with a total molecular mass over 600 kDa and an extremely high efficiency of energy transfer. We solved the structure of the PSI-LHCI supercomplex from pea at 2.8 Å resolution, which revealed the detailed organization of 12 PSI core subunits as well as 4 LHCI subunits, 143 Chl a and 12 Chl b molecules, 35 carotenoids and 10 lipids. Based on the structure, we identified 4 plausible pathways for energy transfer from LHCI to the PSI core.

**ML-03.03.3-001****Structural insights into key events during translation initiation**J. L. Llacer, T. Hussain, B. Wimberly, V. Ramakrishnan  
*MRC-LMB, Cambridge, United Kingdom*

During translation initiation the small ribosomal subunit positions the initiator tRNA and correct start codon of mRNA at the P site with the help of multiple initiation factors. It is known that the initiation pathway requires multiple steps where the ribosome and factors undergo conformational changes. Previously we reported single particle cryo-electron microscopy (cryo-EM) reconstructions of pre-initiation complexes (PICs) from yeast in an open as well as closed conformation of the small ribosomal subunit. These PICs provide insights into key events during eukaryotic translation initiation and roles played by initiation factors, which draws similarity with bacterial initiation.

**ML-03.03.3-002****Structure of viral nucleocapsid by solid-state NMR at 100 kHz magic-angle spinning**D. Cala-De Paeppe<sup>1</sup>, K. Jaudzems<sup>1</sup>, L. B. Andreas<sup>1</sup>, J. Stanek<sup>1</sup>, D. Lalli<sup>1</sup>, A. Bertarello<sup>1</sup>, T. Le Marchand<sup>1</sup>, S. Kotelovica<sup>2</sup>, I. Akopjana<sup>2</sup>, B. Knott<sup>3</sup>, S. Wegner<sup>3</sup>, F. Engelke<sup>3</sup>, A. Lesage<sup>1</sup>, L. Emsley<sup>1,4</sup>, K. Tars<sup>2</sup>, T. Herrmann<sup>1</sup>, G. Pintacuda<sup>1</sup><sup>1</sup>Université de Lyon, Centre de RMN à Très Hauts Champs, Institut des Sciences Analytiques (UMR 5280 – CNRS, ENS

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The atomic-level characterization of large viral particles is one of the greatest challenges of modern structural biology, as well as a fundamental step for the design of effective antiviral treatments. Over the last decades, solid-state NMR (ssNMR) has developed into a powerful structural tool for studying structure and dynamics of solid biological samples at atomic resolution. However, the inherently low sensitivity and poor resolution of the technique has limited its applicability to small proteins that can be tightly packed at a high molar concentration, while large proteins or multi-domain assemblies were mostly inaccessible to site-specific ssNMR studies. This has been recently overcome by the introduction of faster spinning probes, which facilitate the use of proton-detected experiments, which can radically enhance the performance of ssNMR experiments.

Here we demonstrate the effectiveness of the recently developed ssNMR methods employing proton detection at high magnetic field and 100 kHz MAS by structure determination of the 2.5 MDa icosahedral capsid of the AP205 bacteriophage. We show that at this spinning regime spectral resolution is high enough to detect resolved correlations from amide and side-chain protons of all residue types, and to reliably measure a dense network of 1H-1H proximities that define the dimeric capsid subunit structure. The subunit structure is then used in conjunction with a low resolution EM map to construct an atomic-level description of the global capsid architecture.

We expect the approach to enable structure determination for a wide range of molecules such as membrane proteins and macromolecular complexes.

**Tuesday 6 September****9:00–11:00, Hall D****Biochemical mechanisms in tolerance and autoimmunity****S-04.03.3-001****The mosaic of autoimmunity: why we develop autoimmune diseases – the role of environmental factors and especially diet**

Y. Shoenfeld

*Sheba Medical Center, Tel Hashomer, Israel*

Autoimmune diseases are conditions in which the immune system damages normal components of the individual. Initially it was thought that autoimmune disease was the inevitable outcome of the presence of clones of lymphocytes with receptors that recognize self-antigens. Thus tolerance to self, the state of non-autoimmunity, was due to the absence of self-recognizing lymphocytes, the ‘forbidden’ culprits of autoimmune disease. Autoimmune diseases were found to be multifactorial in their etiology. For practical reasons these factors are classified into four categories:

Genetic, which entail the MHC class I, II, and III. A case in point will be the haplotypes of HLA-DRB1 which are prevalent in many classical diseases.

Immune deficiencies: C<sub>1</sub>q, C2, C4 and IgA deficiencies are among the most common defects associated with diverse autoimmune conditions.

Hormonal state, most autoimmune diseases are detected in females at the child bearing ages. The role of estrogens will be delineated. In addition other hormones play a role i.e. prolactin.

Environmental causes: Those are the most important as a trigger factors determining the time and type of disease. They entail infectious agents, chemicals, drugs and even vaccines.

The type of disease in an individual, in an autoimmune prone family, will be determined by the specific combination of the different factors mentioned above.

A special emphasis will be put on Unsaturated fats, salty diet, spicy food and interaction with component of parasites.

**S-04.03.3-003****New potential mechanisms in lupus glomerulonephritis modulation**

A. Doria, M. Gatto, M. Beggio, M. Larosa, F. Saccon, S. Bindoli, L. Iaccarino, A. Ghirardello

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Autoantibodies are key mediators in inducing clinical manifestations of systemic lupus erythematosus (SLE), including glomerulonephritis. The mechanisms by which antibodies may be harmful to self tissues encompass complement mediated inflammation, cell apoptosis and immune-complexes mediated damage, however the precise antibody modulation in SLE has not been unravelled so far.

Lupus nephritis (LN) is a heterogeneous SLE feature characterized by a great variety of symptoms including asymptomatic proteinuria, mild renal disease until end-stage renal failure which are triggered by complex autoantibody interactions.

Novel aspects in the development and self-maintenance of LN have been revealed in recent years, pointing to a multistep inflammatory process which is initiated by anti-chromatin antibodies, including anti-double stranded (ds)DNA and anti-nucleosome antibodies, leading to a self-maintaining inflammatory loop

with spreading of glomerular inflammation. In the maintenance of the inflammatory process pro-inflammatory antibodies are involved, among which anti-C1q seems to play a major role; however, they are counteracted by protective autoantibodies, such as anti-pentraxin 3 antibodies which we have recently reported in association with the absence of LN in humans. In order to verify the protective nature of such antibodies we immunized NZB/NZW F1 mice with pentraxin 3. Mice immunized with pentraxin 3 developed anti-pentraxin 3 antibodies. Notably, immunized mice had delayed proteinuria onset and prolonged survival compared with their littermates.

Thus, several types of autoantibodies exist that play a role in the development and progression of LN. The dynamic balance among pro-inflammatory and protective antibodies is a key point in determining disease course and eventually patient outcome.

**S-04.03.3-002****The phosphorylcholine molecule: the good, the better, the miraculous**J. Omersel<sup>1</sup>, Y. Shoenfeld<sup>2</sup><sup>1</sup>*Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia,* <sup>2</sup>*The Zabudowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel*

Phosphorylcholine (PC), an essential component of sphingo- and phospholipids, not only functions as an important precursor in aminoacids pathways and a regulator of cell physiology, but also serves as a link between human innate and humoral immune response.

In bacteria and helminthes PC functions as a protective mechanism suppressing host's immune system and promoting their survival inside the host. Based on constant challenging of human immune system with PC-epitope it is not surprising that ~10% of the IgM pool is PC reactive. Lately low levels of natural IgM anti-PC were found to be an independent risk factor for cardiovascular diseases.

Moreover, a strong correlation has been reported between high prevalence of helminthes in certain geographic areas and protection from autoimmune and autoinflammatory diseases. Intensive research of the last decade identified a helminthic compound ES-62 as a possible immunomodulator, with a PC-moiety as an important epitope. Screening of small molecule analogues revealed molecules efficiently suppressing development of proteinuria in MRL/Lpr lupus model. In recent experiments a novel compound, tuftsin-PC (TPC), has been shown successful in modulation of Th1/Th2 response. Treatment protocol in murine colitis model prevented severity of colitis with downregulation of IL-1 $\beta$ , TNF $\alpha$ , IL-17 expression and upregulation of IL-10. TPC, given orally or subcutaneously, attenuated collagen induced arthritis in DBA/1 mice and attenuated the development of glomerulonephritis by amelioration of proteinuria. It was confirmed that TPC upregulated anti-inflammatory cytokines, Tregs, Bregs thus providing good therapeutic potential.

As a non-immunogenic biopolymer PC has found its role also in biomedical applications- prosthetics and drug-eluting coronary stents. Its ubiquitous presence from microorganisms to mammals, and doubtless potential from Biochemistry, Pharmacy to clinics, still makes PC interesting for basic and applied research.

**ML-04.03.3-001****Serum vitamin B12 levels in patients with Behçet's disease**E. Paydas Hataysal<sup>1</sup>, A. Unlu<sup>1</sup>, A. Sivrikaya<sup>1</sup>, S. Abusoglu<sup>1</sup><sup>1</sup>Department of Biochemistry, Faculty of Medicine, Selçuk University Konya, Turkey

**Aim:** Behçet's Disease (BH) which is named by the Turkish dermatologist Hulusi Behçet in 1937 is chronic relapsing, systemic vasculitis and autoinflammatory disorder of unknown origin. Vitamin B12 is necessary for hematopoiesis and normal neuronal function. In humans, it is obtained only from animal proteins. In this study, our aim was to compare the level of serum vitamin B12 in Behçet's Disease (BD) patients and healthy control groups.

**Materials and Methods:** Serum samples were collected from 82 healthy control and 86 patients with Behçet's Disease. The mean age for controls and patients were  $34.51 \pm 15.08$  and  $35.23 \pm 15.91$  respectively. Vitamin B12 levels measured with Roche Cobas E170 analyzer. Statistical analysis was performed with SPSS v16.

**Results:** The mean of serum vitamin B12 levels in patients with Behçet's disease ( $336.67 \pm 95.96$ ) were higher but not statistically significant compared to control group ( $354.93 \pm 114.12$ ).

**Conclusions:** Many hypothesis was proposed the etiology of thrombosis and vasculitis in BD. One of those hypothesis its respect to originate from hyperhomocysteinemia. Vitamin B12 deficiency may cause hyperhomocysteinemia. According to this study's results, vitamin B12 levels were not different between BD and healthy groups.

**Tuesday 6 September****9:00–11:00, Hall E****Stem cells and cancer****S-05.03.3-001****Human mesenchymal stromal cells: biological properties and clinical application**

E. Karaoz

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Recent insights into stem cell (SC) biology promise the regeneration of damaged organs. Stem cells (SCs) have the capability of self-renewal and differentiation into a wide range of cell types with various potential clinical and therapeutic applications. SCs are providing hope for many diseases that are currently in need of effective therapeutic methods, including neurodegenerative disorders like; stroke, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and as well as muscular dystrophy disorders like Duchene Muscular Dystrophy and Facio-scapulo-humeral Muscular Dystrophy. For this aim, numerous pre-clinical studies have been achieved and/or in progress on different types of stem cells including, induced pluripotent stem cell (iPS), embryonic stem cell (ESC) and neural stem cells. But there are some complications on the clinical utilization of these cells, due to the reason of ethical issues and especially because of the potential of formation of teratomas via iPSs and ESCs. For this reason, as we glance to the clinical trials ongoing nowadays, we see that mesenchymal stem cells (MSCs) are studied intensely on clinical applications. MSCs attracted particular interest because of their ease of isolation, characterization, apparent multipotency and pleiotropic effects. MSCs are capable of self-renewal and differentiation into specialized cell types and thus have the potential

to promote organogenesis, tissue regeneration, maintenance and repair. Currently, more than 500 human clinical trials employing cell therapy for the treatment of diverse diseases, including cardiac, neurologic, immune and respiratory conditions, are ongoing or completed. The results of clinical trials carried out for the treatment of various diseases, especially including neuro-muscular degenerative disorders, via application of MSCs derived from different tissues and manufactured in our own GMP facility, will be introduced in this presentation.

**S-05.03.3-003****Epigenetic control of stem cell fate and function in the normal and malignant human prostate**

N. Maitland

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Cancer heterogeneity is often the result of separate mutated cell lineages within the same tumour. In prostate cancers, multifocality, catastrophic chromosomal rearrangements and deletions are more characteristic of the genetic landscape. For different epithelial cells within a single clonal lineage, the factors which control phenotypic heterogeneity such as differentiation, and the relationship to therapy responses, is best studied using relevant material from actual patients, and not established cell lines which have silenced many non-essential functions whilst growing in cell culture.

A truly flexible response to microenvironmental influences (such as cancer treatments), is principally achieved in stem-like cells from prostate cancers by epigenetic mechanisms. CpG methylations display a degree of patient specificity, providing an enduring 'off switch' for gene expression, whilst several hormone responses including androgens play an important positive regulatory role. However, rapid activation/expression of small non-coding RNAs can alter chromatin status, and results in widespread synchronous gene expression changes. miRNA expression was more tightly linked to differentiation than pathological status, and the gene ontology term most tightly linked to miRNA expression changes in cancer/stem cells was, perhaps surprisingly, 'response to radiation'. Chromatin status was clearly implicated as a key factor in stem cell fate by the detection of various combinations of modified histones, some indicative of poised chromatin (as found in embryonic stem cells), which also correlated with random monoallelic expression, e.g. of the TMPRSS2-ERG fusion gene.

A combination of epigenetic controls can therefore act flexibly to not only preserve the integrity of normal tissue stem cells, allowing faithful tissue regeneration after injury, but paradoxically also acts to regenerate tumours from stem-like cells after cancer treatments such as radiotherapy.

**S-05.03.3-002****The role of hematopoietic stem cells, cancer stem cells and mesenchymal stromal stem cells for treatment of cancer**

S. Slavin

*Biotherapy International, Tel Aviv, Israel*

Stem cells are undifferentiated normal or malignant cells that can differentiate and produce either more stem cells or differentiate into specialized cells that have well-defined function depending on their source. In the bone marrow there are two types of normal multi-potent stem cells, hematopoietic stem cells and mesenchymal stromal cells (MSC). Hematopoietic stem cells can be used for safer and faster hematologic reconstitution following myeloablative chemo-radiotherapy or for



induction of transplantation tolerance to donor alloantigens that allows durable engraftment of donor lymphocytes for induction of graft-vs.-tumor effects. The other kinds of stem cells present in the bone marrow are MSCs that are multipotent and can be differentiated into cells resembling nearly every tissue. Malignant hematologic diseases and solid tumors too also include cancer stem cells, cancer inducing or cancer initiating cells. Cancer stem cells are *a priori* resistant to available chemotherapy and ionizing radiation and they are considered as one of the reasons why cancer cannot be fully eradicated despite primary response to conventional anti-cancer modalities. Since cancer stem cells are resistant to available anticancer modalities, complete elimination of cancer depends on clinical application of successful anticancer immunotherapy because cell-mediated anticancer cytotoxicity is independent of sensitivity to chemotherapy or radiation. Accordingly, successful treatment of cancer depends on targeting resistant cells with innovative procedures. Taken together, multi-potent stem cells can play a positive or negative role in the course of treatment of patients with cancer: hematopoietic stem cells can help maintain durable engraftment of most effective alloreactive donor lymphocytes, while mesenchymal stromal stem cells can be used for targeting anticancer agents into resistant cancer cells and cancer stem cells using anticancer modalities that can overcome multidrug resistance.

#### ML-05.03.3-001

### Identification of novel hematopoietic small molecules enables *ex vivo* hematopoietic stem cell expansion

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The primary therapeutic modality for many hematopoietic disorders is bone marrow transplantation, which relies on the ability of a small number of hematopoietic stem cells (HSCs) to repopulate all blood cell lineages. However, limited availability of HSCs pose a problem for HSC transplantation from poor mobilizers and umbilical cord blood. In addition, studies aiming to expand gene edited and single cell selected HSCs for the treatment of hereditary blood disorders require development of HSC expansion technologies. We have recently shown that deletion of HSC quiescence regulators in HSC compartment not only leads to cell cycle entry but also HSC expansion. Thus, targeting of HSC quiescence modulators using small molecule inhibitors may provide valuable tools for *ex vivo* expansion of HSCs. To this end, we have determined novel hematopoietic small molecules (HSMs) targeting regulators of HSC quiescence. These HSMs increased murine and human HSC content as shown by *ex vivo* HSC expansion, CFU assays, and *in vivo* HSC analysis in the bone marrow. In addition, they induce HSCs to exit from quiescence state as determined by cell cycle analysis. These studies warrant development of novel HSM induced HSC expansion strategies.

#### ML-05.03.3-002

### Cancer stem cells contribute to the immunosuppressive microenvironment typical of malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a huge medical problem worldwide with a poor prognosis for the poor response to

multimodal therapy and the intrinsic immunoresistance. Indeed, MPM creates a strongly immunosuppressive microenvironment. Tumor-derived stem cells (SCs) are responsible for MPM dissemination and progression, but it is not known if they can also exert immunosuppressive properties. Aim of this work is to investigate if MPM-derived stem cells could be responsible for MPM immunoresistance.

From biopsies and pleural effusions of MPM patients we collected and stabilized MPM cell lines. We isolated the SC component by sorting the SOX2 + Oct4 + Nanog+ ALDHbright cells. In order to confirm functional properties of our MPM-derived stem cells we used clonogenicity and self-renewal assays. High-throughput PCR array was used to examine expression of cytokine genes, JAK/STAT pathway related genes, HMGB1 and ATP release, and calreticulin exposure were used as parameters of immunogenic cell death in response to chemotherapy known for inducing immunogenic effects, such as doxorubicin and cisplatin.

SCs showed higher self-renewal and clonogenicity than non SCs. High-throughput PCR arrays indicated that MPM SCs had higher endogenous expression of immunosuppressive cytokines such as IL-10 and IL-4, and higher expression of JAK/STAT pathway related genes, such as JAK2-3 and STAT3, than non SCs. Doxorubicin and cisplatin induced an immunogenic cell death in non SCs MPM cells, but not in MPM SCs, suggesting that the latter were refractory to the pro-immunogenic effects exerted by chemotherapy.

Overall our study suggested that JAK/STAT axis may be responsible for the immunosuppressive properties of MPM SCs and to the resistance to the immunogenic cell-death induced by chemotherapeutic drugs. Targeting this axis may open new therapeutic possibilities in overcoming MPM immunoresistance.

#### Tuesday 6 September

15:30–17:30, Hall A

### Developments in biomaterials and tissue engineering

#### S-07.01.3-002

### Patient specific material processing approaches in biomaterials and tissue engineering

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**Introduction:** Need for patient specificity of biomaterials and tissue engineering products arises from the fact that the requirement for the therapy is shape dependent. The main developments are currently taking place in this field where the implant needed is specific for the patient because of the form and dimensions. The goal of 3D printed or additively manufactured (AM) biomaterials field is to achieve perfect fit of the implant and the defect site.

**Materials and Methods:** The production method employed depends on the material properties and the need of the defect site. The implant was a biodegradable polymeric meniscus of

poly( $\epsilon$ -caprolactone) designed to perfectly fit the target and degrade in the body in ca. 6 months. Meniscal cells were isolated from the knee of a patient (age 56 years). The cells were seeded on PCL scaffolds and cultured for 14 days. The constructs were visualized with confocal laser scanning microscope (CLSM), microcomputed tomography ( $\mu$ CT) and scanning electron microscope (SEM).

**Results and Discussion:** The implant was prepared in its unseeded form and then human meniscal cells were seeded. It was observed that the construct could be prepared with high fidelity and the human fibrochondrocytes adapted to the surface of the relatively hydrophobic material, uniformly covered the surface of the 3D scaffold and showed the potential of the approach in preparing patient-specific implants.

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### S-07.01.3-001

#### Molecularly designed hydrogels as 3D cellular microenvironments

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New molecularly-designed material structures are required not only to provide the physical and mechanical support to cells, but also to play the role of insoluble and soluble components of the extracellular matrix (ECM). Our research efforts are currently focused on the functionalization of three dimensional hydrogel structures to improve their interaction with biological systems, namely as cell carriers. More efficient cell delivery vehicles are required to fully explore the potential of cell therapies in producing functional substitutes capable of restoring, maintaining or improving tissue function.

We have been investigating the entrapment of several distinct cell types within hydrogels functionalized with cell adhesive, proteolytically sensitive and osteogenic peptidic sequences to modulate cell behavior. Injectable bio-functionalized alginate hydrogels as cell delivery systems were developed. Alginate hydrogels were functionalized with a peptide (PVGLIG) sensitive to proteolytic degradation by matrix metalloproteinases (MMP), and their performance as vehicles for mesenchymal stem cell (MSC) transplantation was analyzed. These hydrogels were further functionalized with an osteoinductive peptide (OGP10-14) to specifically direct MSC differentiation along the osteoblastic lineage. Overall, the ability of the proposed platform to direct the fate of transplanted hMSCs *in loco* was demonstrated, and OGP-releasing hydrogels emerged as a potentially useful system to promote bone regeneration.

The effects of matrix dimensionality and stiffness have also been shown to considerably influence gene expression of entrapped cells in regenerative therapies and in cancer.

### S-07.01.3-003

#### Amphiphilic biomaterials to undertake molecular therapy in cancer

H. Uludag

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Cancers are a group of heterogeneous disorders characterized by abnormal proliferation and migration of cells. Although chemotherapy yields high remission rates in most cancers, most patients eventually relapse due to the proliferation of drug-

resistant cancerous cells. The pharmacological mediator of RNAi, short interfering RNA (siRNA), is currently explored as a highly specific therapeutic agent in several cancers since it provides exceptional specificity for therapeutic intervention. To explore the full potential of siRNA in cancer, we are investigating the utility of lipid-modified, cationic polymers to deliver siRNA and silence desired proteins in breast cancer and leukemic cells. Lipid-substituted amphiphilic polymers are synthesized by modifying polyethylenimines (PEIs) with various lipids, including caprylic, palmitic and linoleic acids. The carriers were self-assembled in aqueous buffers with siRNAs to form nano-sized complexes. While the native cationic polymers did not display cytotoxicity on cells, lipid-grafting on polymers slightly increased the cytotoxicity, which was consistent with increased interaction of polymers with cell membranes. siRNA delivery was dependent on the nature and extent of lipid substitution and different types of lipids were needed for attachment dependent breast cancer and independent leukemic cells. In acute myeloid leukemia cells (AML), caprylic and linoleic acid-substituted polymers performed best among the prepared polymers and gave a siRNA delivery equivalent to better performing commercial reagents. With chronic myeloid leukemia (CML) cells, the same polymers were not effective and we found a particular palmitic acid-substituted PEI to be most effective. The aberrant expression of 'therapeutic' genes was silencing in cells, resulting in a broad range of functional outcomes (e.g., inhibition of migration, cell attachment to bone marrow cells and apoptosis).

### ML-07.01.3-001

#### Nanoparticle-based cancer vaccine to target dendritic cells and the tumor microenvironment

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Few cancer vaccines have been able to lead to an effective tumor regression, which can be explained by immunosuppressive properties of the tumor microenvironment. The elimination of both the tumor itself and the tumor microenvironment, without adversely affecting the desired antitumor effector cells, seems to be an ideal therapeutic strategy to eradicate this disease. Thus, the aim of the study is to develop a nanoparticle (NP)-based cancer vaccine to induce an effective tumor immune response and to silence immune-suppressive cytokines within the tumor site.

Antigen or siRNA-chitosan complexes encapsulated in poly (lactic acid) (PLA) NPs have been formulated by a double emulsion solvent evaporation method. In order to potentiate tumor targeting, NP surface was modified by hyaluronic acid (HA), a targeting moiety that specifically recognizes CD44 receptor. NP size, surface charge and morphology were analyzed by Dynamic Light Scattering, Laser Doppler Electrophoresis and Atomic Force Microscopy (AFM), respectively. Antigen entrapment efficiency (EE) and loading capacity (LC) were quantified by HPLC, while siRNA EE and LC were determined by PicoGreen<sup>®</sup> reagent. Finally, cell viability was determined by Alamar Blue<sup>®</sup> assay. siRNA-NP knockdown capacity is currently under evaluation by Western blotting and flow cytometry.

Overall, NPs presented a mean diameter close to 200 nm with low polydispersity index values, surface charge close to neutrality, and high EE for both antigen and siRNA. NPs were efficiently taken up by DCs using non-targeted NPs and by tumor cells using targeted NP (with HA) and showed no cytotoxic effect on targeted cells and DCs.

Formulation method followed for NP preparation is highly reproducible and this nanoparticulate system constitutes a promising platform for the delivery of tumor-associated antigens and immunomodulators to different cells within the tumor microenvironment.

### ML-07.01.3-002

#### Interaction of human umbilical cord vein derived smooth muscle cells and fibroblasts with natural scaffolds composed of human vascular extracellular matrix proteins

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Tissue engineered small-diameter vascular grafts (VGs) has been preferred instead of autologous blood vessels that exist in limited numbers. Because of the risks of synthetic graft usage natural VGs are considered to be more suitable for clinical use. Extracellular matrix (ECM) proteins and glycosaminoglycans of blood vessels are used to generate tissue engineered VGs.

In this study our first aim is to obtain vascular smooth muscle cells (SMCs) and fibroblasts by differentiate human umbilical cord vein pericytes. For this purpose CD146+ pericytes were isolated from human umbilical cord vein by using collagenase and magnetic activated cell sorting method. After their characterization, they were differentiated to vascular SMCs and fibroblasts. Differentiation was analyzed by immunofluorescent (IF) staining (fibroblast markers; Tenascin-C and collagen type I, smooth muscle cell markers; calponin, caldesmon and alpha smooth muscle actin). Our second aim is to generate natural scaffolds that can mimic vascular layers and to evaluate the vascular cell – matrix interaction. In order to mimic tunica adventitia layer of blood vessels, fibroblasts were mixed with human collagen type I – fibrin gel and to mimic tunica media layer of blood vessels, vascular SMCs were incubated with human collagen type I – elastin – dermatan sulfate gel for 7 days at 37°C. Cell viability and proliferation was analyzed with WST-1. The presence of cells on the scaffolds was shown by IF staining.

According to the results, CD146+ pericytes could successfully differentiate into vascular SMCs and fibroblasts. They were proliferated and maintained their viability on the natural scaffolds composed of human ECM proteins. It is concluded that differentiated pericytes may be considered as an alternative cell source for constructing tissue engineered VGs. The authors wish to thank TÜBİTAK (Project Number: 113S815) for their financial support.

## Tuesday 6 September

15:30–17:30, Hall B

### Aging

#### S-09.03.3-002

#### New insights into the role of mitochondria in age-related neurodegenerative diseases

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Aging is a natural process that is associated with a progressive loss of function of the tissues throughout the organism, albeit at different rates and different levels of sensitivity. The nervous tissue is especially vulnerable to the effects of aging that leads to an impairment of synaptic and cognitive function. Several lines of evidence show that oxidative stress, a sustained increase in intracellular calcium, mitochondrial dysfunction and a compromise of autophagy occur in age-related diseases. In several neurodegenerative diseases for which aging is a risk factor, including Alzheimer's and Parkinson's disease, protein aggregation is accompanied by mitochondria dysfunction. Furthermore, a compromise in the regulation of mitochondria biogenesis and in the control of metabolic processes that relate to insulin resistance and diabetes has also been observed. In this study we will focus on Alzheimer's disease which, in the sporadic form, is the most prevalent form of dementia in the elderly.

Results obtained in human and animal cell cultures exposed to Amyloid  $\beta$  peptides (A $\beta$ ), and also in the Alzheimer's disease triple transgenic mouse model, show that a disruption in calcium and redox cell homeostasis occurs. Simultaneously, mitochondrial dysfunction and an increase in ER stress-induced unfolded protein response were found.

In conclusion, these changes that were observed not only in neuronal cells, but also in endothelial cells, reinforce the involvement of brain capillary endothelium dysfunction in Alzheimer's disease pathogenesis, opening new perspectives in potential preventive therapeutic strategies for this devastating disease.

#### S-09.03.3-003

#### Transcriptomic analysis reveals an outstanding control of apoptosis in centenarians

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Centenarians not only enjoy an extraordinary aging, but also show a compression of morbidity. Using functional transcriptomic analysis of peripheral blood mononuclear cells (PMBC) we identified 1721 mRNAs differentially expressed by centenarians when compared with septuagenarians and young people. Sub-network analysis led us to identify Bcl-xL as an important gene up-regulated in centenarians. It is involved in the control of apoptosis, cellular damage protection and also in modulation of immune response, all associated to healthy aging. Indeed, centenarians display lower plasma cytochrome C levels, higher mitochondrial membrane potential and also less cellular damage accumulation than septuagenarians. Leukocyte chemotaxis and NK cell activity are significantly impaired in septuagenarians compared with young people whereas centenarians maintain it. To further ascertain the functional role of Bcl-xL in cellular aging, we found in transduced lymphocytes from septuagenarians with Bcl-xL a reduction in senescent-related markers. Finally, to demonstrate the role of Bcl-xL in longevity at the organism level, *C. elegans* bearing a gain of function mutation in the Bcl-xL ortholog *ced-*

9 showed a significant increase in mean and maximal life span. These results show that mRNA expression in centenarians is unique and reveals that BeL-xL plays an important role in exceptional aging.

### S-09.03.3-001

#### Genetics and genomics of ageing

J. P. de Magalhaes

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Ageing is the major biomedical challenge of the 21st century, yet it remains largely mysterious, partly because the ageing process involves multiple genes and their interactions with each other and with the environment that remain poorly understood. In this talk, I will present genomic and computational approaches aimed at deciphering the genome and increasing our knowledge about how genes and pathways impact on ageing. Moreover, I will present a systems biology dissection of caloric restriction, a dietary manipulation of ageing, to gather insights into its regulation and identify promising drug targets. We have also been employing whole transcriptome profiling (RNA-seq) to gather insights on ageing and its manipulation by diet. Lastly, I will discuss our recent work in sequencing and analyzing the genome of the longest-lived mammal, the bowhead whale, to identify longevity assurance mechanisms.

### ML-09.03.3-001

#### Neurovascular coupling mediated by neuronal nitric oxide in hippocampus: novel redox modulators

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**Introduction:** The functional connection of glutamate receptors with neuronal nitric oxide synthase in neurons accounts for the coupling between neuronal activation and changes in local cerebral blood flow, i.e., the neurovascular coupling (NVC). We have shown that upon glutamatergic stimulation, nitric oxide (NO) is synthesized and, by diffusing a few hundreds of microns towards neighboring vessels, induces vasodilation via the canonic pathway involving soluble guanylate cyclase. Failure in NVC, either during aging and disease (Alzheimer's disease, AD) or following acute hypoxic conditions, compromises brain integrity and functionality. We have come to conjecture that the redox and functional interplay of NO with ascorbate and nitrite would modulate the functionality of glutamatergic synapses in terms of NVC.

**Methods:** We have used a multimodal approach, comprising microarrays for insertion in the brain of living rodents, consisting of microinjection pipettes, laser Doppler blood flow probes and selective microelectrodes, behavior and biochemical approaches to probe the dynamics and functional impact in terms of NVC coupling of NO, ascorbate and cerebral blood flow *in vivo* in hippocampus of Wistar and Fisher 344 rats and of a mice model of AD.

**Results:** Data support that (1) neuronal-derived NO acts as a direct mediator of neurovascular coupling, (2) neurovascular coupling is impaired in AD and aging due to vascular dysfunction, (3) under acidic/hypoxic conditions, nitrite is reduced by ascorbate to NO and (4) the redox interaction of nitrite/ascorbate/NO contributes to NVC.

**Conclusion:** Overall, these results support a redox cycle of nitrite and ascorbate in the hippocampus, which is translated into NO and CBF transitory increases. Given that nitrite increases NO bioavailability and augments cerebral blood flow in

hippocampus one may envisage that dietary nitrate via the nitrate:nitrite:NO pathway may help sustaining neurovascular coupling in aging and disease.

### ML-09.03.3-002

#### Molecular models of aging: comparative analysis of gene signatures in replicative senescence and stress induced premature senescence

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Senescence is the phenomenon accompanying the cessation of cell division in population of normal diploid cells. It can happen naturally in form of replicative senescence or may be a consequence of external challenges such as oxidative or radiation stress. It is widely accepted that senescence provides protection against possible tumor formation. This research aims to identify gene signatures specific for replicative and stress-induced senescence in human fibroblast cells. To do that, we employed geneXplain bioinformatics software platform on dataset obtained from GSE13330. At the first step, raw data were normalized and the differentially expressed genes (DEGs) were calculated. We found 1410 genes statistically significantly up-regulated in both replicative and stress-induced senescence. 1358 genes are found to be up-regulated specifically in stress-induced senescence, and 1408 genes up-regulated specifically in replicative senescence. Next, DEGs were classified by their functions to dissect the involvement of particular genes in various cell cycle processes and other signaling pathways. At the next step, we applied the upstream analysis approach as a combination of promoter analysis and network analysis. We suggest the role of epiregulin, stromelysin, MGAT5 as the master regulatory molecules in both types of cell senescence. BTEB-2, SHIP-110, SPK, and CDP are identified as master regulatory molecules which are upregulated specifically for stress-induced senescence. IL-1alpha, GM-CSF, MKP-2, MKK3, GDNF, TRIM36, MLTK are identified as master regulatory molecules which are up-regulated themselves specifically in replicative senescence and their genes are part of the gene signature of replicative senescence. Further work on the predicted master regulators and their corresponding networks can help us gain better understanding of the molecular mechanisms of cell senescence, aging and development of cancer.

**Wednesday 7 September**  
**9:00–11:00, Hall A**

**Mechanisms and regulation of protein translocation**

**S-02.04.4-003**

**Genome-wide ribosome profiling of bacterial SRP interaction with nascent polypeptides**

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Signal recognition particle (SRP) is a universally conserved protein-RNA complex that mediates co-translational protein translocation and membrane insertion, by targeting translating ribosomes to membrane translocons. The existence of parallel co- and post-translational transport pathways, however, raises the question of the cellular substrate pool of SRP and the molecular basis of substrate selection. We have determined the binding sites of bacterial SRP within the entire nascent proteome of *E. coli* at amino acid resolution, by sequencing mRNA footprints of ribosome-nascent chain complexes associated with SRP. We find that SRP, based on its strong preference for hydrophobic transmembrane domains (TMDs), constitutes a compartment-specific targeting factor for nascent inner membrane proteins (IMPs) that efficiently excludes signal-sequence containing precursors of periplasmic and outer membrane proteins. SRP associates with hydrophobic TMDs enriched in consecutive stretches of hydrophobic and bulky amino acids immediately when they emerge from the ribosomal exit tunnel. In contrast to current models, N-terminal TMDs are frequently skipped and TMDs internal to the polypeptide sequence are selectively recognized. SRP furthermore binds several TMDs in many multi-spanning membrane proteins, suggesting cycles of SRP-mediated membrane targeting. SRP interaction with nascent chains is not accompanied by a transient slow-down of translation elongation kinetics and is not influenced by the ribosome-associated chaperone Trigger Factor (TF), which has a distinct substrate pool and acts at different stages during translation. Overall, our proteome-wide dataset of SRP binding sites reveals the underlying principles of pathway decisions for nascent chains in bacteria, with SRP acting as the dominant triaging factor, sufficient to separate IMPs from substrates of the SecA-SecB post-translational translocation and TF assisted cytosolic protein folding pathways.

**S-02.04.4-001**

**The non-folding**

**T. Economou**, K. E. Chatzi, A. Tsigotaki, G. Orfanoudaki, M. F. Sardis, M. Koukaki, M. Papanastasiou, M. Trelle, T. J. D. Jørgensen, S. Karamanou, A. Economou  
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Secretory proteins are only temporary cytoplasmic residents. They are synthesized as “preproteins” with signal peptides (SPs)

and cross the bacterial plasma membrane, mainly post-translationally, in non-folded states, before they reach their final destinations and fold. This process overcomes three challenges: sorting from cytoplasmic proteins, avoiding cytoplasmic folding and correct targeting to the membrane-embedded translocase. How preproteins maintain targeting/translocation competence is poorly understood. In contrast to current thinking, we now show that for these processes most preproteins require neither SPs nor chaperones. Preproteins constitute a structurally novel protein class: their mature domains (MDs) remain soluble and yet non-folded/disordered, during cytoplasmic transit. This exposes linear and three-dimensional hydrophobic signals. Together they constitute a “code” that sorts preproteins from cytoplasmic folders, docks them with high-affinity on the translocase independently of SPs. This non-folding “code” is essential for translocation and impacts widely on our understanding of protein trafficking and proteostasis.

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**S-02.04.4-002**

**Cotranslation protein folding inside and outside the ribosome**

**G. von Heijne**

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We have developed a technique based on the use of so-called translational arrest peptides to measure forces acting on a nascent chain during cotranslational processes such as membrane translocation and folding. The technique can be used both *in vitro* and *in vivo*, and can easily be adapted to allow imaging of ribosome-nascent chain complexes by cryo-EM. Recent results on cotranslational folding of cytoplasmic and membrane proteins will be presented.

**ML-02.04.4-001**

**Effects of electro-osmotic flow (EOF) through chitin uptake channel from the marine bacterium *Vibrio harveyi***

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**Introduction:** To date, gene corresponding to chitoporin from the genomic DNA of the marine bacterium *Vibrio harveyi* 650 has been constructed and further purified from Omp8 Rosetta strain to study the functional characterization using BLMs measurements. Chitoporin is an outer membrane protein isolated

from the cell wall of the Gram-negative marine bacteria (*Vibrio* species). We previously demonstrated that chitoporin from *V. harveyi*, namely *VhChiP*, was a trimeric channel, highly selective for chitin uptake.

**Materials and Methods:** Chitoporin; octyl-POE, omp8 rosetta strain; potassium chloride; polyethylene glycol; dextran. In this study, we set out osmotic gradients to explain the effects of the electro-osmotic flow (EOF) on the channel affinity towards its best substrate (chitohexaose).

**Results and Discussion:** Our data suggested that EOF controlled the rate of sugar entry, as well as the residence time of chitohexaose inside the channel lumen. The ion conductivity was significantly reduced from  $2.03 \pm 0.02$  nS to  $1.45 \pm 0.02$  nS when polyethylene glycol (PEG, MW = 35 000 Da) was added on both side of the channel, with the sugar added on the other side. Five-fold and three-fold decreases in the off-rate ( $k_{\text{off}}$ ) were observed under the external applied potential of +100 mV and -100 mV, respectively. On the other hand, four-fold (+100 mV) and five-fold (-100 mV) reduction in the on-rate ( $k_{\text{on}}$ )-rate were observed when PEG was added on the *cis*-side and chitohexaose on the *trans*-side.

**Conclusion:** Such EOF effects on the on-rate and the off-rate clearly demonstrated the channel asymmetry, and strong interaction between chitohexaose and the *VhChiP* lumen, especially when the movement of the sugar molecules was along the direction of EOF.

#### ML-02.04.4-002

### Hemin transmits signals promoting high level transcriptional activation of heme oxygenase-1, cystine/glutamate exchanger, CXC chemokines ligand 1 (CXCL1) and CXCL8 in human erythroleukemia K562 cells

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Heme, a complex of Fe (II) with protoporphyrin IX is an essential component of many hemoproteins carrying out vital functions (gas transport, cell respiration, bioenergetics and erythro-differentiation, sensing just to mention a few). Heme however, released from damaged RBCs during intra/extravascular haemolytic disorders can act catastrophically. We used hemin (an oxidised form of heme) at 50  $\mu$ M to uncover the mechanism via which hemin transmits signals leading to hemin-induced cell death (HICD).

K562 cells exposed to hemin were assessed for cell growth kinetics, cell death and selective transcriptome changes as well as translocation of the major transcription factor (TF) NF-E2-related factor 2 (Nrf2). qPCR analysis of several genes was complemented by Western blot analysis to track the nuclear translocation of Nrf2 TF.

Thus far, our results show that hemin: a) caused cell cycle arrest first (via induction of p27 protein) and then cell death, b) promoted release of Nrf2 TF from its anchor Kelch-like ECH associated protein 1 (Keap-1) in cytosol and translocation into the nucleus within 2 h exposure, c) induced selective transcriptional activation of *Heme oxygenase-1 (HO-1)*, *Cystine/glutamate exchanger (xCT)* and *Glutamate-cysteine ligase, catalytic (GCLC)*, all driven by Nrf2. (d) Moreover, hemin induced extensively the transcription of genes encoding the chemokines *CXCL8* and *CXCL1/2*, while slightly affected the transcription of genes encoding *IL-1B*, *IL-6*, and *TNF $\alpha$* , all driven by NF- $\kappa$ B.

Whether hemin-induced transmitted signals involves nuclear translocation of other TFs in addition to Nrf2, is under investigation.

In conclusion, these data indicate for first time that heme transmits signals mediated by TF nuclear translocation and subsequent activation of transcription of genes involved in stress response, metabolism and angiogenesis.

S.K.G. was a recipient of Onassis and IPEP Foundations for post-graduate studies.

#### Wednesday 7 September

9:00–11:00, Hall B

### Human microbiome (microbiota)

#### S-02.06.4-001

### Systems biology of microbiomes

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Changes in the human gut microbiome are associated with several chronic diseases. So far, the vast majority of studies have sought to identify community-wide compositional shifts and link these to disease etiology but have largely failed to establish functional linkages. We have recently established a framework to systematically characterise the structure and function of microbiota in relation to human physiology. More specifically, we have developed methods for integrated multi-omic analyses to resolve attributes of gastrointestinal microbiota at either the population- or community-level. We have for example applied our methodologies to samples collected over time from families with multiple cases of type 1 diabetes (T1DM). Despite the absence of consistent taxonomic differences across families, T1DM-specific effects in the microbiome were apparent at the functional level. These results suggest that functional differences expressed by microbiomes may ultimately be key to our understanding of the role of the gut microbiome in chronic diseases. Furthermore, the results allow formulation of hypotheses which posit how expressed functions may affect human physiology. To test such hypotheses we have recently developed a modular, microfluidics-based model, HuMiX, which allows co-culture of human and microbial cells under conditions representative of the gastrointestinal human-microbe interface. HuMiX-based human cell cultures recapitulate *in vivo* transcriptional, metabolic and immunological responses in human intestinal epithelial cells following their co-culture with gut microbiota grown under anaerobic conditions. Thereby, HuMiX allows new insights into a range of fundamental research questions linking the gastrointestinal microbiome to human health and disease. In summary, the establishment of these novel methodologies will allow us to unravel the exact molecular mechanisms of human-microbial interactions in the context of human health and disease in the future.

#### S-02.06.4-002

### Microbiome analysis of the human gut and the ocean

P. Bork

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The human gut microbiome cannot be readily studied using metagenomics and harbours more than 1000 species that are associated with important functions but also with more than 30 human diseases. I will illustrate the diagnostic potential of microbial marker species using colon cancer as an example

(Zeller et al., *Mol.Sys.Biol.*, 2014), but will also describe emerging global patterns, such as the existence of microbial community types at genus level, which we dubbed enterotypes (Arumugam et al., *Nature*, 2011), indicating a stratification of the human population. At the strain level, our gut microbiota appears much more individualistic (Schloissnig et al., *Nature*, 2013) and we can thus trace, for example, colonization after faecal microbiota transplantation (Li et al., *Science* 2016), or pinpoint specific commensal or pathogenic strains in stool samples. Human pathogens are mostly coming from the environment and thus it is crucial to study biodiversity of microbes world-wide to understand their spreading patterns. The feasibility of such global approach is illustrated by the TARA oceans project surveying the microbial diversity of this vast ecosystem by studying of plankton at a truly planetary scale (Bork et al., *Science* 2015 and refs therein).

### S-02.06.4-003

#### Microbiota-dependent signals link ATF6-driven cell stress to colonic tumorigenesis

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Activation of the endoplasmic reticulum unfolded protein response (erUPR) contributes to the pathogenesis of inflammatory bowel diseases (IBD), and might increase the risk of developing colorectal cancer (CRC). However, there is a lack of mechanistic evidence for a causative role of erUPR in oncogenic tissue transformation. Activating transcription factor 6 (ATF6) mediates one branch involved in sensing and signaling of the erUPR. To address the role of ATF6-mediated erUPR signaling in intestinal epithelial cells (IEC), we generated Villin-Cre-driven IEC-specific transgenic mice overexpressing the activated form of ATF6 (nATF6IEC).

Homozygous mice (tg/tg) demonstrate spontaneous colonic adenoma formation, independent of inflammation, with an incidence of 100% at 12 weeks of age. Western Blot analysis of human colon cancer tumors suggests high levels of nuclear ATF6 to be significantly associated with an increased risk for disease recurrence. High-throughput 16S-rRNA gene sequencing of caecal microbiota showed a clear separation of bacterial communities according to the tumor-promoting genotype. Furthermore, a reduced bacterial diversity was already evident at pre-tumor stages in tg/tg mice. Loss of mucin-filled goblet cells was associated with increased microbial penetration of the mucus barrier. GF housing of tg/tg mice was shown to prevent tumor formation, despite the presence of activated erUPR. Antibiotic treatment induced a shift in microbial composition, and antagonized hyperproliferation and tumor incidence. Most importantly, microbiota transfer into GF recipients reestablished tumors in tg/tg mice, clearly demonstrating a causative role of bacterial communities in colonic adenoma formation.

Our newly generated mouse model demonstrates that microbiota-derived signals are integrated into activated erUPR of the epithelium to cause colonic tumors, suggesting that ATF6-signaling serves as a risk factor for the spontaneous formation of CRC and IBD-associated CRC.

## Wednesday 7 September

9:00–11:00, Hall C

### Single molecule techniques – Applications in biology

#### S-03.04.4-001

#### The enigmatic role of lipids in membrane protein interactions

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The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Specifically, it is unclear to what extent membrane proteins are affected by their immediate lipid and protein environment and vice versa. I will show in three examples, how lipids behave in the live cell plasma membrane:

(i) We developed a special method termed TOCCSL, which allows for determining molecular associations of diffusing membrane proteins based on single molecule brightness analysis. By this method, we found the homo-dimerization of GPI-anchored proteins in the live cell plasma membrane<sup>1</sup>.

(ii) A micropatterning approach was used in combination with single molecule tracking to quantify the influence of a glycosylphosphatidylinositol-anchored protein (GPI-AP) – a typical marker of liquid ordered phases – on its molecular environment directly in the live cell plasma membrane<sup>2</sup>. We found that the captured proteins merely acted as bulky obstacles to the diffusion of other membrane constituents, but did not influence their membrane environment over distances past their actual physical size. Our results imply that the outer leaflet of the plasma membrane is in a homogenous single phase regime under physiological conditions.

(iii) Finally, we analyzed the association behavior of the human serotonin transporter. We found stable oligomerization at the plasma membrane<sup>3</sup>, but exchange of subunits at the ER membrane. Our data indicate phosphoinositides-mediated kinetic trapping of the SERT subunits.

#### References

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#### S-03.04.4-002

#### Membrane receptor nanoclustering as functional unit of immune cells: from nanoscopy to single molecule dynamics

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Organization by compartmentalization is a general property of natural systems that efficiently facilitates and orchestrates biological events in space and time. In the last decade, compartmentalization of the plasma membrane of living cells has emerged as a dominant feature present at different spatiotemporal scales and regulating key functions of immune cells. I will discuss two important glycoproteins expressed on the surface of various human antigen-presenting cells (APCs), those spatiotemporal organization regulate their function: DC-SIGN and CD1d. DC-SIGN is a pathogen recognition receptor expressed on dendritic cells playing a decisive role in stimulation of different immune responses. Using a combination of multi-color single particle tracking and super-resolution microscopy approaches we investigated the effect of DC-SIGN molecular structure on the

formation DC-SIGN nanoclusters and their dynamic interaction with other molecular components of the cell membrane, including the glycocalyx matrix, lipid nanodomains and clathrin coated pits. We find that DC-SIGN compartmentalizes at different spatiotemporal scales and that this hierarchical organization is crucial to facilitate its pathogen binding capabilities and clathrin-dependent endocytosis. In the second example, I will focus on CD1d, a non-classical MHC protein, involved in the presentation of lipid antigens to T cells. Using a combination of advanced biophysical techniques we found that CD1d molecules organize in nanoclusters on the membrane of APCs. We further discovered that the actin cytoskeleton prevents enhanced CD1d nanoclustering by hindering physical encountering between CD1d diffusing nanoclusters, reducing basal activation of invariant natural killer T (iNKT) cells. Our results indicate that regulation of CD1d nanoclustering through the actin cytoskeleton constitutes a novel mechanism to fine-tune peripheral iNKT cell autoreactivity.

### S-03.04.4-003

#### Lessons from observing single RNPs in living cells

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We analyzed the mobility of single fluorescently labeled ribosomal and messenger RNA-protein complexes (rRNPs and mRNPs) in live cell nuclei and obtained detailed insight into their intranuclear dynamics. Even large RNPs travel within a few seconds from transcription sites to the nuclear pore complexes (NPCs) in the nuclear envelope. The motion is not purely diffusive, but binding or retardation occur frequently. The NPCs mediate nuclear transport of all cargo across the nuclear envelope. The molecular details of RNA translocation through the NPCs are not yet completely deciphered. One major obstacle to address this question is the visualization of RNA molecules without interfering with their native behavior. We have explored several ways to solve this problem. The ATP-dependent RNA helicase Dbp5 is a key component of mRNA export at the NPC and interacts with both mRNA and the NPC protein Nup214. However, the underlying molecular mechanism is quite unclear. We use single molecule enzymology to scrutinize the sequence of Dbp5 interactions with RNA/ATP, Gle1/IP6 or Nup214 and their respective reaction kinetics. We aim at resolving the question: What exactly happens at the cytoplasmic exit of the NPC during export, and how is the directionality of the mRNA transport accomplished?

### ML-03.04.4-001

#### Single-particle fluorescent microscopy analysis of nucleosomes and their complexes with linker histone H1.5

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Inter-nucleosomal linkers are functionally important DNA regions involved in chromatin folding and function. Linkers interact with

histones H1 and H5, which mediate formation of higher order chromatin structures, as well as with other proteins that regulate transcription. Detailed studies of interaction between linker-containing nucleosomes and linker histones are carried out to decipher the principles of chromatin structural organization and regulation.

To use a single particle fluorescence microscopy in these studies, a fluorescently-labeled DNA templates were designed, and uniquely positioned mononucleosomes with 40-base pair DNA linkers were assembled. The linkers were labeled with donor-acceptor pair of Cy3 and Cy5 fluorophores that allowed analysis of the inter-linker distance in nucleosomes using Förster resonance energy transfer (FRET) effect.

Single particle FRET (spFRET) microscopy of freely diffusing nucleosomes revealed co-existence of two equally probable states of nucleosomes with different linker conformations: open one with FRET efficiency (E) of 6% and semi-closed one with E of 37%. Binding of histone H1.5 to nucleosomes was detected at nanomolar concentrations of H1.5. The complexes had relatively fast association and slow dissociation rates. H1.5 binding brought nucleosomal linkers together and produced a homogeneous population of complexes with E of 73%.

Designed mononucleosomes are fluorescent sensors that are highly sensitive to the conformation of linkers. spFRET microscopy and the developed mononucleosomes are currently used for the structural analysis of complexes between nucleosomes and different chromatin architectural proteins. This work was supported by the grant 14-24-00031 from Russian Science Foundation.

### ML-03.04.4-002

#### Investigating cell membrane dynamics by STED-FCS and spectral imaging

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Molecular interactions in the plasma membrane of living cells are key in cellular signalling. Protein-protein or protein-lipid complexes, the formation of lipid nanodomains (often denoted as "membrane rafts"), or diffusional restrictions by the cortical cytoskeleton are considered to play a functional part in a whole range of membrane-associated processes. The direct and non-invasive observation of such interactions in living cells is often impeded by principle limitations of conventional far-field optical microscopes, specifically with respect to limited spatio-temporal resolution. We present how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution stimulated emission depletion (STED) nanoscopy with fluorescence correlation spectroscopy (STED-FCS) and spectral imaging.

## Wednesday 7 September

9:00–11:00, Hall D

### Molecular mechanisms of inflammation

#### S-04.04.4-003

#### PTX3 as a mechanism of regulation of inflammation and cancer-related inflammation

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Innate immunity consists of a cellular and a humoral arm. Tumor-associated macrophages are a key component of tumor-promoting inflammation. The long pentraxin PTX3 as originally



cloned (cDNA and genomics, mouse and human) as an IL-1 inducible gene. We have used the long pentraxin PTX3 as a paradigm for the humoral arm of innate immunity and its interplay with cells. PTX3 is a multifunctional soluble pattern recognition receptor characterized by a C-terminal domain highly homologous to C-reactive protein and serum amyloid P component, associated to a N-terminal domain unrelated to other known proteins. PTX3 is produced upon stimulation with proinflammatory cytokines and Toll-like receptor engagement most prominently by monocytes/macrophages. The molecule binds complement components and microbial moieties. It mediates effector function via Fcγ receptor and complement. Recent results suggest a function at mucosal surfaces. PTX3 plays non-redundant functions including innate immunity against selected microorganisms and regulation of inflammation. In addition PTX3 plays a role in the interplay between the cellular and the humoral arm of innate immunity. PTX3 deficiency was associated with increased susceptibility to mesenchymal and epithelial carcinogenesis.

PTX3 expression was epigenetically regulated in selected human tumors (e.g., leiomyosarcomas and colorectal cancer) by methylation of the promoter region and of a putative enhancer. Thus, PTX3, an effector molecule belonging to the humoral arm of innate immunity, acts as an extrinsic oncosuppressor gene in mouse and man by regulating Complement-dependent, macrophage-sustained, tumor promoting inflammation.

#### S-04.04.4-002

### Tissue macrophages – development and functions

S. Jung

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Macrophages are myeloid immune cells that are strategically positioned throughout the body tissues, where they ingest and degrade dead cells, debris and foreign material, and orchestrate inflammation and immune defense. Defying earlier notions, most tissue-resident macrophage compartments are established prenatally and develop locally, alongside their host tissue and independent from each other. Maintenance of macrophage compartments relies on longevity and self-renewal and is in most tissues independent from ongoing hematopoiesis. Rather, substantial postnatal replacement of the embryonic populations by adult monocyte-derived cells seems restricted to selected tissues, such as the heart and gut, linked to unique homeostatic challenges of organs. Tissue macrophages are hence, aside from immune sentinels, integral components of their host tissue and tailored to contribute to tissue homeostasis. Factors governing local adaptations are emerging and tissue specialization is also prominently reflected in discrete gene expression profiles and epigenetic signatures. Moreover, recent studies have revealed defined roles of tissue macrophages to organ development and homeostasis. Specific contributions of macrophages to steady state organ function remain for most tissues however incompletely understood.

Here I will discuss our recent efforts to dissect macrophage differentiation and tissue specialization and report on a new role of adipose tissue macrophages in the control of tissue innervation.

#### S-04.04.4-001

### Essential role of the NFAT signaling pathway in microbial-induced inflammation

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Innate immunity is the most ancient form of response to pathogens and it relies on evolutionary conserved signaling pathways, i.e. those involving the NF-κB pathway. Nevertheless, increasing evidence suggests that factors that have appeared more recently in evolution, such as the Nuclear Factor of Activated T cell transcription factor family (NFATc), also contribute to innate immune response regulation in vertebrates. We have observed that exposure to inflammatory stimuli, such as LPS, induces the activation of NFATc factors in innate immune cells, including conventional dendritic cells (DCs), with a mechanism TLR4-independent involving instead CD14. In turn, NFAT contributes to the regulation of inflammatory cytokine production, antigen transport to the lymph nodes via the lymph and DC life cycle. The NFAT-controlled phenomena and the consequences of NFAT activation will be discussed in models of microbial infections and sterile inflammation.

#### ML-04.04.4-001

### The impact of helicobacter – stimulated IL-10 producing regulatory B cells on T cell differentiation

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B cells have been associated with regulation of excessive inflammation in models of arthritis, contact hypersensitivity, type I diabetes, *Salmonella* infection and colitis. In mice, multiple subsets of IL-10-producing Breg cells were described, including transitional 2 marginal-zone precursor (T2-MZP) cells, CD5<sup>+</sup>CD1d<sup>hi</sup> B (B10) cells, marginal-zone (MZ) B cells, and Tim-1<sup>+</sup> B cells. Using mouse models of infection with *H. felis*, a close relative of the human gastrointestinal pathogen *H. pylori*, we found that B cells activated by *Helicobacter* TLR-2 ligands produce IL-10 and induce IL-10-producing CD4<sup>+</sup>CD25<sup>+</sup> T regulatory-1 (Tr-1)-like cells both *in vitro* and *in vivo* (Sayi et al, JI, 2011). Tr-1 conversion depends on TCR signaling and a direct T-/B-interaction through CD40/CD40L and CD80/CD28. B and Tr-1 cells cooperatively acquire suppressive activity *in vitro* and suppress excessive gastric *Helicobacter*-associated immunopathology *in vivo*. Our recent data suggest that, *H.felis* stimulated IL-10 producing B cells exhibit a heterogeneous population showing mainly CD1d<sup>hi</sup>CD5<sup>+</sup> regulatory B10 cell phenotype that shares phenotypes with T2-MZ B cells. In addition to that, stimulation of B cells with *H. felis* leads to both IgM and IgG2b secretion from IL-10<sup>-</sup> B cells, but not from IL-10<sup>+</sup> B cells. Next, we investigate the role of *Helicobacter* – stimulated (*Hstim*) IL-10<sup>+</sup> B cells and IL-10<sup>-</sup> B cells on CD4<sup>+</sup> T cell differentiation. For that purpose, *Hstim* IL-10<sup>+</sup> B and IL-10<sup>-</sup> B cells were placed with CD4<sup>+</sup> T cells. Flow cytometry results indicated that IL-10<sup>+</sup> B cells induce Tr1 cell differentiation. Also, our findings show, for the first time, that IL-10<sup>-</sup> B cells induce Th17 cell differentiation as well as Tr1 cells. Overall, our data contributes to the characterization of *Hstim*-IL-10<sup>+</sup> B and IL-10<sup>-</sup> B cells and also to define the impact of *Hstim* B cells on the differentiation of CD4<sup>+</sup> T cells.

**ML-04.04.4-002****The intracellular pyrimidine 5'-nucleotidase NT5C3A is a negative epigenetic player during interferon and pro-inflammatory cytokine response****K. Khabar***King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia*

Pyrimidine 5'-nucleotidase (NT5C3A) is an enzyme that is involved in nucleoside catabolism and is highly inducible during interferon response (IFN). Although known as an erythrocyte-specific enzyme, its role in non-hematopoietic cells or during IFN is elusive. Here, we identify that NT5C3A is an IFN-regulated gene involved in cytokine suppression by negative regulation of the NF- $\kappa$ B pathway. First, we identified NT5C3A in cells of different types that are stimulated by various inducers, including Type-I IFN and TNF. Treatment of human epithelial and fibroblast cells with Type-I IFN led to the induction of NT5C3A expression in a temporal- and a dose-dependent manner. This process is mediated by the JAK1-STAT signaling pathway and requires largely IRF-1 expression. We demonstrated a novel role for NT5C3A acting as an intracellular antagonist of the inflammatory cytokine response. Over-expression of NT5C3A but not the pathogenic mutants in the  $\alpha/\beta$  Rossmann-like domain catalytic unit suppressed IL-8 (CXCL8) expression. The absence of NT5C3A augmented the expression of TNF-activated IL-8 and reduced the IFN-mediated suppression of IL-8. NT5C3A-mediated attenuation of TNF-induced IL-8 involves chromatin remodeling at the IL-8 promoter and requires both the induction and activation of nuclear SIRT6 through an increase in NAD<sup>+</sup> levels. This process caused deacetylation of the lysine K9 and K14 of histone H3. We identified a marked reduction of H3K14 acetylation at the nucleosome near the transcription start site of the IL-8 promoter. NT5C3A-mediated activation and accumulation of nuclear SIRT1 deacetylase during TNF-stimulation suppressed the level of acetylated lysine 310 of the RelA/p65 co-accessory unit of NF $\kappa$ B, leading to transcriptional repression. Overall, the study pinpoints a novel anti-inflammatory IFN-mediated pathway, which involves functional NT5C3A enzyme catalytic activity and describes an important negative feedback regulator of the TNF-signaling pathway.

**Wednesday 7 September****9:00–11:00, Hall E****Functional genomics and proteomics****S-08.01.4-001****The Monkey King and Pigsy ferrying the proteomic sutras in the 3rd millenium: a chronicle****P. G. Righetti***Politecnico di Milano, Milano, Italy*

In the saga *Journey to the West* (16th century, Ming Dynasty) the Monkey King (a divinity in the Chinese Olympus) helps the Buddhist monk Xuanzang (Tripitaka) to retrieve and bring back to China the sacred Buddhist texts (the sutras), against evil forces contrasting his mission. Out of metaphor, the superpower that ferried the proteomics sutras in the third millennium is

definitely mass spectrometry (MS), especially the family of Orbitrap machines. Yet, notwithstanding the extraordinary power of modern MS, plenty of low-abundance proteins (LAP) escape detection and identification as they are buried underneath the high abundance species (HAP), which swamp their weak signals. Going back to the saga, another divinity helped Tripitaka to escape evil forces on his way back to China: Pigsy, half a man, half a pig, whose main connotation was tremendous avidity for food. So, in the proteomics field, the second divinity helping detection of LAPs is certainly the combinatorial peptide ligand library (CPLL) methodology, which is here presented. CPLLs, like Pigsy, have a tremendous avidity for LAPs and enhance their signal up to four orders of magnitude.

I will present here some outstanding results obtained when analysing a few biological fluids such as the cytoplasm of the red blood cells, where as many as 1578 species have been identified, as well as of the human cerebrospinal fluid and sera. In other mammalian fluids the harvest has also been bountiful: in goat's milk, 452 unique proteins have just been identified, vs. a handful only a while ago. In bovine colostrum, in which 85% of the species are immunoglobulins, 1786 proteins have now been catalogued, an exorbitant number, indeed. Among them a set of 93 proteins involved in wound healing process was identified and clustered on the basis of different biological functions.

Boschetti, P.G. Righetti, *Low-Abundance Proteome Discovery. State of the Art and Protocols*, Elsevier, Amsterdam, 2013, 341 pp.

**S-08.01.4-003****Understanding venom variability: a challenge ahead and a meeting point for evolutionary biologists and antivenom producers****J. J. Calvete***Instituto de Biomedicina de Valencia, CSIC, Structural and Functional Venomics Lab, Valencia, Spain*

Adaptive radiation involves the diversification of a lineage into species that differ in phenotypic traits used to exploit different resources. Ecological opportunities arise when new or previously inaccessible resources are encountered or are newly exploitable following the acquisition of a key evolutionary innovation. Venom is a polygenic complex adaptive trait. Inter- and intraspecific venom variability has long been appreciated by herpetologists and toxinologists. Although the molecular mechanisms that generate this diversity remain largely elusive, recent evidence indicate that genetic and postgenomic factors are at play. Understanding the molecular basis of venom evolvability demands qualitative and quantitative comparisons of the temporal and spatial patterns of venom variation. Research on venoms has been continuously enhanced by advances in technology. The combination of venom proteomics, particularly top-down venomics, and venom gland RNAseq enables the generation of comprehensive locus-resolved venom proteome maps, and will revolutionize proteoform-resolved venom analysis in the coming years. Recent venom and antivenomic studies across a number of lineages will illustrate how genus-wide venomics aids in the identification of global evolutionary trends across the phylogeny of snakes that may guide in the generation of polyspecific antivenoms of broad therapeutic use.

**S-08.01.4-002****Epigenetic maintenance of germ cell identity by the conserved SET1/MLL complex**F. Palladino<sup>1</sup>, F. Beurton<sup>2</sup>, M. Caron<sup>2</sup>, C. Bedet<sup>2</sup>, A. Knutson<sup>3</sup>, A. Rechsteiner<sup>3</sup>, S. Strome<sup>3</sup>, V. Robert<sup>2</sup><sup>1</sup>*Ecole Normale Supérieure de Lyon/Université Lyon, Lyon, France*, <sup>2</sup>*Ecole Normale Supérieure/Université de Lyon, Lyon, France*, <sup>3</sup>*University of California Santa Cruz, Santa Cruz, United States of America*

Maintenance of totipotency is critical for germline identity, and relies on the activity of several mechanisms, including epigenetic regulation. Expression profiling carried out in our lab previously showed that inactivation of the conserved SET1 histone H3 Lys4 (H3K4) methyltransferase in *C. elegans* results in ectopic expression of somatic genes in the germline, resulting in germline transdifferentiation. Interestingly, conversion of germline cells to soma only becomes apparent after several generations, suggesting that additional changes in gene expression over subsequent generations may contribute to the transdifferentiation process.

To identify the pathways driving transdifferentiation, we are using high-throughput sequencing to look at how the germline transcriptome changes between subsequent generations in the absence of SET1 activity. In parallel, we are using proteomics to identify additional partners of the SET1 methyltransferase complex, and using genetic approaches to study how these contribute to preserving germline identity.

Altogether, these complementary genome-wide approaches will shed light on how epigenetic regulation preserves germ cell identity. Because depletion of SET1 complex components results in differentiation of embryonic stem cells, H3K4 methylation mediated by SET1/MLL complexes may more generally constitute a conserved cellular machinery involved in preserving stem cell pluripotency.

**ML-08.01.4-002****Mass spectrometric detection of chondroitin sulfate proteoglycan 4 nitration in patients with acute pulmonary embolism**O. H. Ozturk<sup>1</sup>, C. Eken<sup>2</sup>, Z. Avci<sup>1</sup>, F. Ozcan<sup>1</sup>, M. Aslan<sup>1</sup><sup>1</sup>*Department of Biochemistry, Akdeniz University Medical Faculty Antalya, Turkey*, <sup>2</sup>*Department of Emergency Medicine, Akdeniz University, Antalya, Turkey*

Pulmonary embolism (PE) is a common cardiovascular emergency and affects a large number of patients. Acute PE-induced oxidative stress can lead to the accumulation of specific nitroproteins that may play a role in disease progression. The impact of nitration of a single tyrosine residue often has broad implications on the activity of biologically critical proteins, which has become increasingly related to pathological conditions. In this study, we used a proteomic approach to analyze nitrated serum proteins in patients diagnosed with acute PE and healthy controls. Nitrotyrosine (NO<sub>2</sub>Tyr)-containing proteins were immunoprecipitated from serum with a NO<sub>2</sub>Tyr affinity sorbent. Precipitated proteins were separated by SDS-PAGE and visualized by Coomassie Blue staining and Western blotting with mouse monoclonal anti-NO<sub>2</sub>Tyr antibody. Among the numerous immunoreactive bands observed in disease patients, the 250 kDa protein band was in-gel digested and analyzed by MALDI-TOF mass spectrometry (MS). Mass fingerprint data sets obtained from the peptide fragment ions matched human chondroitin sulfate proteoglycan 4 (CSPG4\_HUMAN) with Mascot algorithm analysis giving a score of 57 ( $P < 0.05$ ). Chondroitin sulfate proteoglycan 4 [PDB Id: Q6UVK1] in the serum is a C1q inhibitor (C1q INH) ranging in size from 21 to 750 kDa. The C1q INH has been described in

terms of its ability to precipitate C1q and inhibit its ability to initiate the complement cascade. Chondroitin sulfate proteoglycan 4 also modulates the plasminogen system by enhancing plasminogen activation, an enzymatic cascade involved in the control of fibrin degradation. Nitration-induced alterations of CSPG4 activity can thus possibly lead to decreased fibrin degradation and enhanced complement system activity. Given these considerations, future studies are aimed understand the relevance of NO<sub>2</sub>Tyr modifications in CSPG4 relating to changes in protein structure and function.

**Wednesday 7 September****15:30–17:30, Hall A****Cell cycle and circadian clocks****S-02.10.4-001****Metabolic and redox oscillations in the circadian clockwork**

A. Reddy

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Much is now understood about the nature of transcriptional oscillations in multiple organisms. Until recently, however, little was known about non-transcriptional mechanisms that may contribute to circadian timekeeping. Our recent work in red blood cells and marine algae pointed towards redox oscillations playing an unanticipated role in 24 h timekeeping. A family of proteins called the peroxiredoxins appear to be key readouts of the non-transcriptional clockwork, and their circadian oscillation is, remarkably, conserved in all phylogenetic domains, including bacteria, archaea and eukaryotes. Thus, redox mechanisms are likely to be deeply embedded within the clockwork of multiple species, in stark contrast to the lack of evolutionary conservation of transcriptional components of the clockwork. These new insights have led to a new model of circadian clock regulation, with reciprocal connectivity between metabolism and transcriptional processes that form the clockwork.

**S-02.10.4-003****Resonating circadian and cell cycle oscillators in single mammalian cells**

F. Naef

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Circadian and cell cycles are two periodic, noisy, cell-autonomous processes with a period in the range of 1 day. Consequently, when these cycles run in parallel in the same cell, their coupling may lead to resonances or even synchronization. Observations of circadian variations in mitotic indices in mammalian cells and on the daytime-dependence of cell division in mouse liver have led to the hypothesis that the circadian cycle might gate cell-cycle progression. A better understanding of how the two systems mutually interact is currently of great interest, notably to better understand the role of circadian clocks in proliferating tissues such as the epidermis, immune or stem cells.

Our recent quantitative time-lapse imaging study of circadian cycles in dividing mammalian fibroblasts clearly indicated that both oscillators tick in a tightly synchronized state. Moreover, contrary to our expectations, we found that the cell cycle progression exerts a unilateral influence on the circadian clock, and not the opposite. I will describe recent development to

reconstruct the full stochastic dynamics of the two interacting cycles. This allows us i) to make more specific predictions on precise cell cycle events that influence the circadian clock, such as the condensation of chromosomes, or the dilution of cellular components ensuing divisions; ii) test those prediction using further markers of cell cycle events. While studying crosstalk between different cellular systems has been an important theme in systems biology, the coupled oscillator system exhibits rich low dimensional phenomenology that can be efficiently understood in terms of the inferred non-linear dynamics.

### S-02.10.4-002

#### MYC inversely coordinates the circadian clock with cell growths and proliferation

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The circadian clock and the cell cycle are major systems controlling expression of large sets of genes in temporal fashion. It seems conceivable that the potentially conflicting programs are coordinated. We show that overexpression of the oncogene MYC in U2OS cells attenuates the circadian system and conversely promotes cell growth and proliferation while downregulation of MYC strengthens the circadian clock and reduces proliferation. MYC shares common binding sites with the circadian transcription factor BMAL1 and attenuates BMAL1-mediated activation of E-box-dependent clock genes. However, the inhibition of the circadian clock is crucially dependent on the formation of repressive complexes of MYC with MIZ1 and subsequent downregulation of the core clock genes *BMAL1* and *CLOCK*. Similarly, MYC-dependent stimulation of proliferation of U2OS cells requires the interaction of MYC with MIZ1. Furthermore, *BMAL1* and *CLOCK* expression levels correlate inversely with MYC levels in human lymphomas. Our data suggest that MYC acts as a master coordinator that inversely modulates the mutual impact of the cell cycle and the circadian clock on gene expression.

### I-001

#### Circadian regulation of oxygen levels

Y. Adamovich<sup>1</sup>, B. Ladeux<sup>1</sup>, M. Golik<sup>1</sup>, M. Koeners<sup>2</sup>, G. Asher<sup>1</sup>  
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Our body has an internal timekeeping system hardwired in the genome named the ‘‘Circadian Clock’’. This clock has an endogenous pace of about 24 h, and hence the name ‘circadian’ (from Latin ‘around a day’). The mammalian timing system generates circadian rhythms that help organisms predict and adapt to environmental changes caused by the light/dark cycle. It is achieved by creating daily rhythms in physiology and behavior, including gene expression, body temperature, rest-activity and feeding-fasting cycles. Tissue oxygenation is an important physiological signal in all aerobic life. In order to meet energetic needs, oxygen must be supplied to virtually all cells. It is well known that metabolism exhibit circadian rhythmicity, and we hypothesized that oxygen levels should be rhythmic as well. Indeed we show in animals that oxygen exhibit daily variations on multiple levels: rate consumption of oxygen in mice cages, oxygen concentrations in mice blood, and oxygen levels in rat tissues. The amount of oxygen consumed and its

levels in blood and in tissue peaked during the dark/active phase and decreased during the light/resting phase. We further show that disrupting these daily oxygen cycles by exposing mice to decreased oxygen levels (hypoxia) allow them to adjust their internal time faster when challenged with a 6 h shift in their normal light/dark regimen (jetlag). The speed in which circadian clocks adjust their time in response to light perturbation is an important measurement of the clock plasticity. At the molecular level, we demonstrate that decreased oxygen can induce the expression of core clock genes composing both the negative and the positive arms of the pacemaker. Overall, our results validate the existence of daily oxygen rhythms, and suggest that oxygen signals back to the circadian clock.

### ML-02.10.4-002

#### The role of circadian clock genes in metastatic lung cancer: the potential of per1 for early diagnosis

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Lung cancer is the leading cause of death from cancer worldwide, and the metastatic spread of lung tumors is even more dangerous than the primary tumors. Recently, circadian clock genes, which generate daily rhythms in metabolism and physiology, have attracted attention as important players in cancer development. Therefore, we examined the expression of circadian clock genes and circadian clock regulated genes at different stages of carcinogenesis to consider the circadian clock affects metastasis in patients with lung cancer.

We analyzed the circadian clock and target genes by qPCR in non-small cell lung cancer samples. Formalin-fixed paraffin-embedded specimens were used to isolate RNA from samples, which were classified as normal lung tissue ( $n = 23$ ), primary tumors ( $n = 23$ ), and lymph-node metastases ( $n = 23$ ) according to pathological investigation.

The most significant change in circadian clock gene expression was in *Per1*. The expression decreased as tumor stage advanced and its expression was 5-fold lower in metastatic samples. This finding was in correlation with previous studies suggesting *Per1* as a tumor suppressor gene. Interestingly, we also found a good correlation in expression between *Per1* and the cell cycle inhibitor gene *p21*, such that a decrease in both *Per1* and *p21* was found in metastatic samples relative to primary tumors.

The suggested role for *Per1* as a possible tumor suppressor gene is also supported by our analysis. The expression of *Per1* is gradually lost during tumor progression and reached its lowest levels in metastases. While *p21* expression did not decrease more in metastases and *Per1* expression continued decreasing more in metastases which suggests that *Per1* might have metastasis-related functions independent of the cell cycle. In addition, the abnormal expression levels of *Per1* in metastatic tumors could be a potential marker for early diagnosis and may help the development of new therapeutic strategies in patients with lung cancer.

**Wednesday 7 September**  
**15:30–17:30, Hall B**

**Chemical and biochemical aspects of oxidative stress**

**S-09.04.4-003**

**Physiological glycation by methylglyoxal, glyoxalase 1 and developments in disease mechanisms and clinical therapeutics**

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The glyoxalase system consists of two enzymes, glyoxalase 1 (Glo1) and glyoxalase 2, and reduced glutathione, catalyzing the conversion of methylglyoxal (MG) to sequentially S-D-lactoylglutathione and D-lactate. It is the primary enzymatic defense in cells against damaging glycation of proteins and nucleotides by MG. Major MG-derived glycation adducts of protein and DNA are hydroimidazolone MG-H1 and imidazopurinone MGdG, respectively.

Accumulation of MG – “dicarbonyl stress” – occurs in ageing and metabolic and vascular disease. It is linked to both increased formation of MG and down-regulation of Glo1, with consequent increased protein damage and dysfunction by dicarbonyl glycation. Dicarbonyl stress is mild in obesity, moderate in diabetes and severe in renal failure. Experimental studies suggest a role in insulin resistance and inflammation of obesity. Increased formation of MG occurs in hyperglycaemia associated with diabetes and Glo1 is down-regulated in the kidney, retina and nerve. Dicarbonyl stress thereby contributes to the development of diabetic nephropathy, retinopathy and neuropathy. Experimental and clinical studies also indicate Glo1 deficiency is a driver of cardiovascular disease.

Transcription of Glo1 is under stress responsive control via transcription factor Nrf2. This provided a strategy to develop inducers of Glo1 expression. We recently completed a clinical trial of a Glo1 inducer formulation in overweight/obese subjects. The Glo1 inducer increased expression and activity of Glo1, decreased plasma MG and total body MG-protein glycation. It decreased fasting and postprandial plasma glucose, decreased insulin resistance and improved arterial dilatation. It also decreased vascular inflammation and produced minor weight loss and improved kidney function. The Glo1 inducer is a suitable treatment for improved metabolic and vascular health in overweight and obese populations and also for diabetes and related vascular complications.

**S-09.04.4-002**

**Pharmacological applications of modulators of oxidative stress**

L. Saso

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Oxidative stress is linked with many pathologies ranging from cancer to neurodegenerative diseases and antioxidants are presumably of therapeutic value in such diseases. Different direct and indirect mechanisms by which natural antioxidants exert their action, including scavenging and metal chelating effects, mimicking the antioxidant enzymes or upregulation of their

expression, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and inhibition of pro-oxidant enzymes among others will be presented.

Some of the causes of the failures of antioxidant therapies in clinical trials will be illustrated. Novel approaches to antioxidant therapy that include mitochondria-targeting drugs, antioxidant gene therapy and approaches for improvement of cell uptake and alteration of subcellular compartment localization will be described. In the end, “shadows” that are shortcomings of antioxidant therapy as well as “lights” that include positive outcomes will be addressed.

It is concluded that if we learn from failures, invest on agents with higher potential and take advantage of novel emerging approaches, antioxidants could be an asset for the management of some of the carefully chosen oxidative stress-related diseases.

**References**

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**S-09.04.4-001**

**Natural redox modulators of oxidative stress and chemoresistance in cancer therapy: beneficial versus deleterious effects**

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The roles of oxidative stress in physiology and pathology have been intensively studied over the last decades, but the problem is still far beyond our full comprehension. The roles of free radicals and antioxidants have been entirely redefined recently. Free radicals widely recognized as absolute evils causing damage to biologically important molecules and structures, have been recently transformed into positive actors, in the appreciation of their essential impact in the intracellular signaling and regulation of apoptosis. In contrast, the great hope that antioxidants could be the panacea resolving practically many health problems has vanished, due to the growing number of inconclusive or negative data from studies. Multiple drug resistance (MDR) may develop against chemotherapeutic agents with unrelated chemical structure and mechanism of action used for the treatment of cancer, reduces the efficacy of drugs, and remains as a major challenge in the treatment of cancer. A complex redox pattern underlies MDR problem. Natural product modulators of MDR are used as low toxicity chemosensitizers to enhance the efficacy of anticancer protocols and to overcome MDR. Redox active drugs could provide a valid and promising way to overcome MDR in cancer therapies via targeting an axis consisting of drug transporters, aryl hydrocarbon receptor, phase I/II metabolic enzymes, and the inducible Nrf2-linked pathway. The mechanism underlying the MDR inhibition by natural products obtained from plants and fungi lies in the blockade of the drug binding site, interference with the ATP hydrolysis process, alteration in integrity of cell membrane lipids, and decrease in Pgp or/and MRP1 expression. During coadministration, natural modulators compete with cytotoxic agents for binding to the active site of the transporters and reduce drug efflux. However, beneficial versus deleterious effects of these substances must be well evaluated in chemoresistance and cancer therapy.

**ML-09.04.4-002****Serum glycomarkers of endoplasmic reticulum stress and lysosomal-endosomal functional disturbances in cardiovascular diseases**I. Pysmenetska<sup>1</sup>, T. Butters<sup>2</sup><sup>1</sup>*Dnipropetrovsk Medical Academy, Dnipropetrovsk, Ukraine,*<sup>2</sup>*CarboNet Consulting Ltd, Oxford, United Kingdom*

Ischemia and hypertension cause stress of intracellular organelles leading to a disruption of their functions. The hallmark of the endoplasmic reticulum stress is alteration of protein homeostasis. This organelle triggers unfolded protein response to restore cellular homeostasis, in particular through activation of endoplasmic reticulum-associated degradation (ERAD) of misglycosylated and/or misfolded proteins. ERAD is one of the main intracellular sources of free oligosaccharides (FOS), unbound structural analogues of glycans of glycoconjugates. The lysosomal-endosomal degradation of glycoconjugates is a different source of their appearance. FOS structures and their alterations may reflect functional status of these organelles. Free oligosaccharides in plasma obtained from patients with cardiovascular diseases, before and after standard treatment, were investigated to evaluate this idea.

After plasma deproteinization and FOS purification the oligosaccharides were labeled with anthranilic acid, separated into the neutral and charged fractions with ion-exchange chromatography. FOS were analysed using high-performance liquid chromatography (HPLC).

HPLC profiles of FOS revealed a changing pattern of heterogeneity, depending on the severity of the disease. Three main enlarged glycan species in the neutral fraction and one peak in the charged fraction distinguished the FOS of the patients from those of the healthy volunteers. After treatment, the spectrum changes were observed in neutral fractions. The depth of these changes had individual features but a full profile recovery was not observed. There was no impact of the treatment on the charged fraction. That might indicate a stress prolongation of endosomal-lysosomal system in spite of the therapy.

The study of free oligosaccharides of blood plasma is a new field of Glycobiology allowing a non-invasive evaluation of an organism state at the level of the cell organelle functional status in norm and different diseases.

**Thursday 8 September**  
**9:00–11:00, Hall A**

**Intracellular organization****S-02.05.5-001****Microfilament dynamics and role in cell morphogenesis and migration**

D. Louvard

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The apex of intestinal epithelial absorptive cells is covered by a remarkable array of well-ordered finger-like membrane protrusions supported by an organized bundle of actin filaments. These microvillus arrays are settled on a complex terminal web area. Together they form the brush border, a functional unit specialized for secretion and absorption of salts and nutrients. Rather than a structural entity of enterocytes, the brush border represents a crucial dynamic interface that modulates gut homeostasis. This exceptional structure implies the existence of precise regulatory mechanisms to control its assembly and dynamics. We will focus on molecular determinants involved in the regulation of

enterocyte brush border morphogenesis. In addition, we will discuss the consequences of brush border misorganization and loss of integrity during wounding or due to pathogenic, inflammation and genetic disorders.

**S-02.05.5-002****Biomechanical control of tissue morphogenesis**

A. Munjal, S. Kerridge, A. Jha, V. Paduano,

A. Garcia de las Bayonas, T. Lecuit

<sup>1</sup>*IBDM Aix-Marseille University & CNRS, Marseille, France*

Epithelial tissues exhibit a remarkable dual property of robustness and fluidity. This operates on different time scales and relies on unique mechanical properties of the cell cortex and on adhesive interactions between cells. We seek to understand the fundamental molecular mechanisms responsible for this property.

To that end we develop a range of approaches, from the genetic and pharmacological perturbations of molecular components, the quantitative imaging of proteins using a variety of photonic methods, probing of the physical properties of cells within intact tissues, and computational modelling of morphogenesis at different scales (molecular to tissue scales).

I will present our recent progress in understanding how adhesion and cortical tension control the dynamic remodelling of cell contacts in the primary epithelium of *Drosophila* embryos. I will also report recent findings delineating a novel GPCR signalling pathway responsible for the spatial regulation of cortical tension by the Rho1 pathway during tissue invagination and tissue extension.

**S-02.05.5-003****VE-cadherin modulates YAP intracellular localization and signalling**C. Giampietro<sup>1</sup>, A. Disanza<sup>1</sup>, G. Scita<sup>1</sup>, E. Dejana<sup>1,2</sup><sup>1</sup>*IFOM, FIRC Institute of Molecular Oncology, Milan, Italy,*<sup>2</sup>*Uppsala University, Rudbeck Institute, Uppsala, Sweden*

Besides promoting endothelial cell-to-cell adhesion, Vascular Endothelial (VE)-cadherin transfers intracellular signals contributing to vascular haemostasis. Signalling through VE-cadherin requires association and activity of different intracellular partners including beta catenin, p120 and many others. YAP/TAZ transcriptional co-factors are important regulators of cell growth, organ size, contact guidance and *intercellular junction* organization.

We found that EPS8, a signalling adapter regulating actin dynamics and the architectural organization of the cytoskeleton, is a novel partner of VE-cadherin and is able to modulate YAP transcriptional activity. By biochemical and imaging approaches in cultured endothelial cells we found that EPS8 associates to VE-cadherin in early confluent monolayers, when junctions are under remodelling.

Eps8 exerts a dual role: i) It binds to VE-cadherin and, by increasing its turnover, causes inhibition of PI(3)K-signalling and prevents, in this way, YAP phosphorylation and inactivation; ii) It directly interacts with  $\alpha$ -catenin competing for the binding of the latter protein to 14-3-3/YAP complex, thus promoting YAP translocation to the nucleus and transcriptional activation. Junctional association of YAP inhibits nuclear translocation and inactivates its transcriptional activity both *in vitro* and *in vivo* in Eps8 null mice. Collectively, our data identify novel components of the adherens junction complex and introduce a new molecular mechanism through which AJ complex controls YAP transcriptional activity.

**ML-02.05.5-001****Cholesterol-dependent alterations in synaptic vesicle fusion and exocytotic glutamate release in vitamin D deficiency**

L. Kasatkina, I. Triakash

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Neurotransmission is governed by the life cycle of synaptic vesicles (SVs) which store neurotransmitters and deliver them to the synaptic cleft. Neurotransmitter uptake, vesicle trafficking and membrane fusion are highly dependent on membrane lipid composition. This study aimed to track the Ca<sup>2+</sup>-dependent SV fusion and glutamate release in brain nerve terminals in vitamin D deficiency and after enrichment/depletion of membrane cholesterol.

Studies were conducted on rat brain nerve terminals and in cell-free system: isolated SVs interacting with target membranes in homo- or heterotypic manner. The techniques included lipid analysis (liquid chromatography), registration of intracellular pH, membrane fusion (R18 lipid probe dequenching) and endogenous glutamate release.

Vitamin D deficient rats displayed increased cholesterol level in blood cells and plasma membranes (PMs) of cortical nerve terminals. The increase of cholesterol level in synaptic PMs by 27% in vitamin D deficient rats was associated with the higher cholesterol level in SV membranes. Elevation of cholesterol level in nerve terminals suppressed the depolarization-induced exocytotic glutamate release by 16 ± 3%. Furthermore, the intentional reduction/increase of cholesterol level in PMs and SVs with acceptor methyl-β-cyclodextrin attenuated both homo- and heterotypic membrane fusion.

There is correlation between high cholesterol level in presynaptic PMs and blood cells in vitamin D deficiency. High cholesterol level in presynaptic membranes disturbs homo- and heterotypic membrane fusion. The last may be one of the reasons of impaired depolarization-induced exocytotic glutamate release in vitamin D deficiency.

Vitamin D is implicated in cholesterol metabolism in brain and beyond. The level of PM cholesterol in vitamin D deficiency as well as the enrichment/depletion of synaptic PM cholesterol strongly impact cholesterol level in SVs and substantially modify the membrane fusion and exocytotic glutamate release.

**Thursday 8 September****9:00–11:00, Hall B****Extracellular matrix and metalloproteinases****S-02.07.5-003****Insights into extracellular regulation of growth factor signalling from nanostructural approaches**

C. Bayley, H. Troilo, A. Barrett, M. Lockhart-Cairns, R. Dajani, T. Jowitt, R. Collins, C. Baldock

*University of Manchester, Manchester, United Kingdom*

The mammalian tolloid family of metalloproteinases is essential for tissue patterning and extracellular matrix assembly. The four members of the family: bone morphogenetic protein-1 (BMP-1), mammalian tolloid (mTLD), tolloid-like (TLL)-1 and TLL-2 differ in their substrate specificity and activity, despite sharing similar domain organization. Additionally, the mode of action of regulators, such as Twisted gastrulation (Tsg) which enhances

tolloid cleavage of chordin to regulate BMP growth factor signalling, is as yet undefined.

TLL-2 and Tsg were purified for use in chordin cleavage assays and binding studies performed using surface plasmon resonance. Their oligomeric state was defined using multiangle light scattering followed by X-ray scattering and/or electron microscopy to characterise their structures.

Here we show that TLL-2, the least active member of the tolloid family, is predominantly monomeric in solution. Structural and biophysical analyses reveal an elongated shape and flexibility in the absence of calcium. Furthermore, we show that TLL-2 can cleave chordin *in vitro*, similar to other mammalian tolloids, but truncated forms of TLL-2 mimicking BMP-1 are unable to cleave chordin. However, N- and C-terminal non-catalytic domains from all mammalian tolloids bind chordin with high affinity.

Although Tsg and tolloids are involved in chordin cleavage, no interaction was detected between them suggesting that Tsg functions by inducing conformational change in the substrate chordin making it more susceptible to tolloid cleavage. Substrate exclusion by dimerization has explained differences in the activities of monomeric BMP-1 and dimers of mTLD and TLL-1 but it appears unlikely that substrate exclusion via dimerisation is a mechanism for regulating TLL-2 activity. Therefore the mechanisms underlying substrate specificity and activity in the tolloid family are complex with variation between family members and depend on both multimerisation and substrate interaction.

**S-02.07.5-002****Collagen sensing: how do discoidin domain receptors transmit a signal across the membrane?**

B. Leitinger

*Imperial College London, London, United Kingdom*

The discoidin domain receptors, DDR1 and DDR2, are receptor tyrosine kinases that are activated by a number of different collagen types. Aberrant DDR signalling contributes to a number of diseases, such as fibrotic disorders, arthritis and cancers. The DDRs are attractive novel drug targets, but little is known about how collagen binding results in DDR activation.

The DDRs form ligand-independent, constitutive dimers and therefore do not follow the canonical model of receptor tyrosine kinase activation (i.e. ligand-induced dimer formation). In this talk I will outline our current understanding of how DDRs transmit a signal into the cell, based on our biochemical, crystallographic and cell imaging approaches.

**S-02.07.5-001****Syndecan proteoglycans: gatekeepers of the cell adhesion phenotype**

J. Couchman

*University of Copenhagen, Copenhagen, Denmark*

The syndecans are transmembrane heparan sulphate proteoglycans with a long evolutionary history. While mammals possess four syndecan genes, invertebrates have one which is expressed in nervous and other tissues. The mammalian syndecans are widespread and almost all cells express at least one of the family. Work by many laboratories over the past 25 years has shown roles for syndecans in a range of processes, including the regulation of cell adhesion, actin cytoskeleton and migration. Mis-expression has been noted in many diseases, including vascular and musculoskeletal diseases as well as various cancers. All syndecans can link to the actin cytoskeleton, and in some cases promote junction formation. Since the phenotype of a

single syndecan gene knockout is mild in the mouse, we hypothesized that there is redundancy between family members, but no molecular basis for this had been established. Through the use of microarrays, genetic experiments in the mouse and *C. elegans*, and imaging, we have established that a major function of invertebrate and vertebrate syndecans is the regulation of a class of calcium channel. The transient receptor potential channels of the canonical type (TRPC) are widespread regulators of resting calcium levels and are responsive to stretch activation. Their role in regulating cell adhesion is exemplified by syndecan-4 in fibroblasts. Primary wild type fibroblasts establish microfilament bundles (stress fibres) terminating at focal adhesions. Matching syndecan-4 null cells mostly lack these structures. We established that resting calcium levels in the null cells was higher than the wild type cells. We could eventually show that by deleting the TRPC7 channel in the null cells, a wild type phenotype was obtained. This and other evidence suggests that syndecan-4 controls the TRPC7 channel to maintain a calcium level consistent with stress fibre formation. Further genetic experiments showed that neuronal guidance in *C. elegans* is also partly regulated by the syndecan control of TRP-1 and -2 channels, precise orthologs of the mammalian channels. We therefore propose that the syndecan-TRPC axis is a conserved regulator of the cell adhesion phenotype with impact on migration.

#### ML-02.07.5-001

### Visfatin promotes a disintegrin and metalloproteinase with thrombospondin motifs-5 gene expression through p38 MAPK and NF- $\kappa$ B pathways in human chondrocytes

K. O. Yaykasli

Department of Medical Biology, Kahramanmaraş Sütçü İmam University, Medical Faculty, Kahramanmaraş, Turkey

Visfatin also called NAMPT (Nicotinamide phosphoribosyltransferase) and PBEF1 (pre-B-cell colony-enhancing factor 1) is adipokine (adipocytokines), and characterized in 1994. Visfatin with inflammatory feature involves in normal physiology and pathological problems. The underlying putative reasons of inflammation in arthritic diseases such as rheumatoid arthritis (RA), osteoarthritis (OA) still needed to explain. Obesity is considered to be risk factor for RA and OA. It was clarified that visfatin expression was increased in obesity and RA. The upregulation mechanisms of Matrix Metalloproteinase-2 and -9 by visfatin in several cell types were shown before. So, catabolic effect of visfatin on articular cartilage makes visfatin central place in the investigation of arthritic diseases. Visfatin caused induction of ADAMTS-5 (aggrecanases-2) was investigated by our group. However, this induction mechanism was not clarified yet. For this aim, human chondrocytes was preincubated with p38 (SB203580) and NF- $\kappa$ B (QNZ) inhibitors for 30 min. Then, 500 ng ml<sup>-1</sup> visfatin was added the cell culture medium, and incubated for 6 h additionally. At the end of the incubation isolated total RNA was reverse transcribed, and the gene expression level of ADAMTS-5 measured using RT-PCR (real-time polymerase chain reaction) method. As a results, visfatin caused ADAMTS-5 upregulation was almost inhibited by p38 and NF- $\kappa$ B inhibitors. This result has vital importance to understand the mechanisms of the obesity caused-inflammation in arthritic diseases. In conclusion, the aggrecanase induction pathways may become a target to develop new therapy for arthritic diseases.

#### ML-02.07.5-002

### Shear force sensing of the epithelial Na<sup>+</sup> channel (ENaC) involves glycosylated asparagines and the extracellular matrix

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Mechanotransduction describes how cells translate mechanical forces into cellular signals. The epithelial Na<sup>+</sup>-channel (ENaC) formed by  $\alpha$ ,  $\beta$  and  $\gamma$  subunits is regulated by shear force (SF) and contributes to electrolyte/fluid-homeostasis and blood pressure regulation. The mechanisms how ENaC senses SF are unknown.

Human  $\alpha\beta\gamma$  ENaC was expressed in *Xenopus* oocytes and SF-activated currents were recorded with the two-electrode voltage-clamp technique. The contribution of the extracellular matrix (ECM) for SF-activation of ENaC was addressed by degradation of the ECM with hyaluronidase. Site directed mutagenesis was performed to replace asparagines in the extracellular domain of  $\alpha$  ENaC to reveal their potential function as tethers for SF sensing. Pressure myography was performed to assess the role of the ECM for shear force sensing of ENaC in isolated and intraluminally perfused carotid arteries from mice.

SF-dependent activation of ENaC was observed in *Xenopus* oocytes (increased transmembrane current) as well as in carotid arteries by an augmented vasodilation in response to the ENaC inhibitor amiloride. The SF effects were decreased after hyaluronidase treatment in both *Xenopus* oocytes and arteries, indicating that an intact ECM is required for SF sensation of ENaC. Replacement of *N*-glycosylated extracellular asparagines in the  $\alpha$  ENaC subunit (N312 and N511) was observed to reduced the SF-activation of ENaC. This indicates that specific *N*-glycosylated asparagines are involved in SF sensation by providing a connection to the ECM.

Our results uniquely identify the ECM and extracellular *N*-glycosylated asparagines as crucial components for SF sensing of ENaC. This provides new insights into the mechanism of SF sensation of ENaC suggesting that the ECM and the *N*-glycosylated asparagines form/are part of an extracellular tether that connects the channel subunit with the ECM. This could also be a new, yet unidentified mechanism for mechanotransduction.

#### Thursday 8 September

9:00–11:00, Hall C

### Plant biochemistry and molecular biology

#### S-02.08.5-003

### Harnessing plant metabolic diversity

A. Osbourn

John Innes Centre, Norwich, United Kingdom

Plants produce a wealth of natural products that are valuable as industrial or pharmaceutical products. The vast majority of the natural product diversity encoded by plant genomes remains as yet untapped. The explosion in available plant genome sequence data coupled with affordable DNA synthesis and new DNA assembly technologies now offer unprecedented opportunities to harness the full breadth of plant natural product diversity and generate novel molecules in foreign hosts using synthetic biology approaches. The recent discovery that genes for the synthesis of different kinds of natural products are organised in biosynthetic gene clusters in plant genomes is now opening up opportunities for systematic mining for new pathways and chemistries. The



production of plant and plant-inspired molecules in heterologous plant and microbial expression systems will enable the development of rational strategies to produce known and new-to-nature chemicals that are tailored for particular applications. This presentation will focus on our work on triterpene engineering using synthetic biology approaches.

### S-02.08.5-002

#### Nature's palette: biosynthesis, regulation and metabolic engineering of betalain pigments

A. Aharoni

Weizmann Institute of Science, Rehovot, Israel

Betalains are tyrosine-derived red-violet and yellow pigments found in plants only of the Caryophyllales order, which hold both scientific and economic values. Their pH in-dependence and high stability make them a natural pigment of choice for food industries, in which they are widely used as natural food colorants. Their strong antioxidative activities have prompted research into their potential health-promoting properties and led to commercialization of a variety of betalain-based dietary supplements. Elucidation of the first step in the betalain biosynthetic pathway has enabled us to engineer stable betalain production in tobacco and additional plant species through heterologous expression of three genes taking part in the fully decoded betalain biosynthetic pathway. These plants serve as an exceptional tool to study the putative roles of betalains in plants, particularly in resistance to biotic and abiotic stress cues. In my presentation I will also highlight several strategies that could be applied to crop plant protection and the production of high-value plant products through engineering the betalain pathway and its intermediates.

### S-02.08.5-001

#### Valeriana officinalis as a novel platform for plant natural product drug discovery

J. Chappell

University of Kentucky, Lexington, United States of America

Valerian is a nutraceutical preparation from the roots of *Valeriana officinalis* that is commonly recommended for relief of tension, anxiety and insomnia. The greatest biological efficacy of valerian has been correlated with freshly harvested and carefully dried root preparations, and with the iridoid alkaloid and sesquiterpene content of these preparations. The valepotriates are epoxyiridoid esters with the dominant species being valtrate. Because of putative instability and water insolubility of the valepotriates, some investigations have suggested that the sesquiterpene compounds are more important for the biological activity of valerian. To determine which chemical components of *V. officinalis* are important for its biological activities, we have developed several important capabilities and tools to support this effort. First, we have developed and relied upon transcriptomic and metabolomic resources to identify the genes encoding for these unique biosynthetic capabilities. Second, the methodology for genetic engineering hairy root cultures of *V. officinalis* having diverse chemical profiles has been developed. Third, we have been working on the development of a novel test platform for assessing the anxiolytic activity of the various engineered hairy root culture lines. Progress in all of these areas will be presented.

### ML-02.08.5-002

#### The multistress regulator translocator protein affects cellular energy homeostasis by enhancing lipid droplets breakdown: evidence from plant and yeast cells

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Translocator proteins (TSPOs) are evolutionary conserved  $\alpha$ -helical membrane proteins. The non-essential TSPO is implicated in various human diseases such as inflammation and anxiety disorders. Although the atomic resolution structures of TSPOs from different species were achieved recently, their physiological role remains murky and controversial. TSPOs are expressed or up-regulated under stressful conditions. Plant TSPOs are temporarily induced by water deficiency and constitutive expression of the *Arabidopsis* TSPO (AtTSPO) can be detrimental to the plant cell. Regulatory degradation of AtTSPO by the plant cell requires heme binding and an active autophagic pathway.

We used biochemical, imaging, and pharmacological approaches to demonstrate that expression of TSPO enhances the breakdown of lipid droplets (LD) in plant and yeast cells, resulting in altered energy homeostasis. Constitutive expression of AtTSPO resulted in a drastic reduction of triacylglycerol (TAG) as compared to the wild type (WT) plant. Conversely, AtTSPO knockout mutant plant accumulated about 20% more TAG than the WT plant. Constitutive expression of the point substitution H91A, which is deficient in heme binding and is more stable, had no effect on LD content. Interestingly, heterologous expression of AtTSPO and its H91A variant in *Saccharomyces cerevisiae* (devoid of a TSPO homologue) showed similar results in terms of LD levels. *Schizosaccharomyces pombe* and its TSPO null mutant were used to show that TSPO enhances LD consumption. Indeed, as compared to the wild type strain, the null mutant contained more TAG, diacylglycerol and esters, and was more sensitive to cerulenin, a fatty acid and sterol biosynthesis inhibitor, suggesting that TSPO promotes the breakdown of LD.

Taken together, our findings suggest that TSPO regulates the level of energy reserves in the cell, and the TSPO-dependent breakdown of cytoplasmic LD may be linked, at least in the plant cell, to degradation of TSPO.

**Thursday 8 September  
9:00–11:00, Hall D**

### Personalized medicine

### S-08.02.5-002

#### Translational proteomics for precision medicine: if genomics is a photograph, proteomics is a movie

C. Borchers

University of Victoria, Victoria, Canada

While the genome provides a blueprint for proteins, proteins are the functioning units in cell signaling events. Protein abundances may differ from genomic information due to complex regulatory events that occur during gene transcription and translation. Stratifying cancer patients, for example, based on genomic markers has had only limited success. The discrepancies between genomic and proteomic data in tumors may partly explain why genome-

based biomarkers and novel targeted drugs have resulted in only modest improvements in patient response.

Mass spectrometry is now poised to make major contributions to precision medicine in two different areas. The first area is that of biomarker discovery and validation. Large-scale quantitation projects including biomarker validation – long the “bottleneck” in the biomarker pipeline – are now feasible due to advances in quantitative proteomics, particularly the development and standardization of highly-multiplexed and accurate quantitative proteomics methods such as multiple reaction monitoring (MRM) for the simultaneous targeting of large numbers of analytes.

The second area is that of the quantitation of biomarkers after they have been identified. This quantitation must be done in a clinically usable, sensitive, precise, and accurate manner. With this in mind, my laboratory has been developing a variety of automated immuno-matrix assisted laser desorption/ionization (iMALDI) assays, which can provide extremely rapid quantitation of targeted analytes using instrumentation already widely available in clinical laboratories. One of our iMALDI projects involves quantitation of the expression levels and phosphorylation stoichiometry of phosphorylation sites in AKT1 and AKT2, proteins in the PI3K/AKT/mTOR pathway, which is one of the most commonly dysregulated pathways in cancer development. AKT is over-expressed in various cancers, and AKT is currently the target of several novel inhibitors currently in clinical trials.

### S-08.02.5-003

#### Quantitative proteomics based on high-resolution accurate mass (HRAM) analysis – application to biological and clinical questions

B. Domon

University, Luxembourg, Luxembourg

Targeted analyses using parallel reaction monitoring (PRM), performed on high-resolution and accurate-mass (HRAM) mass spectrometers, present the selectivity and sensitivity to confidently quantify peptides in complex samples. The internal standards (IS) used for isotope dilution quantification were recently leveraged to actually drive the acquisition (“internal standard triggered-PRM”, IS-PRM [1]), thus increasing the scale of screening experiments while ensuring high sensitivity. Here, the IS-PRM technique has been evaluated in the context of additional proteomics experiment types.

First, it has been explored in combination with fast liquid chromatography separation to progress analytical throughput. The IS-PRM method was particularly suited to this configuration, which required maximized acquisition efficiency to maintain a decent number of peptides analyzed in each analysis. The developed setup enables the quantification of up to 50 peptides in 100 samples within 1 day.

In addition, the performance of the technique was further investigated with respect to quantification accuracy. Several options were evaluated, including standard curve and single-point calibration methods, to assess accuracy, robustness and throughput. A novel analytical workflow was designed, combining the concurrent addition of multiple isotopologous internal standards and the IS-PRM acquisition, to improve the accuracy of quantitative analyses.

Third, the field of application of the IS-PRM technique has been expanded to include the analysis of post-translational modifications. The approach was explored for the determination of phosphorylation site occupancy on a large scale. These developments will also be applicable to other types of post-translational modifications, as illustrated with the analysis of asparagine deamidation products (aspartate/isoaspartate).

### S-08.02.5-001

#### Integration of tissue and urine proteomics for biomarker discovery and verification of urological cancer

Y. T. Chen

Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan

Bladder cancer is a common urological cancer. A noninvasive, inexpensive, and highly sensitive/specific bladder cancer marker would be helpful in improving diagnosis, decreasing patient morbidity, and/or lowering costs associated with surveillance cystoscopy. Urine, urinary microparticles, and bladder tissue samples are attractive materials for bladder cancer biomarker discovery. We have applied isotopic labeling coupled with liquid chromatography-tandem mass spectrometry to discover bladder cancer biomarkers in urine and tissue samples isolated from control and bladder cancer patients. Multiplexed multiple-reaction-monitoring (MRM) assay offers a useful workflow for the development of a urinary biomarker panel.

A DAVID analysis of dysregulated tissue proteins reveals that exposure to toxic substances will contribute to the increased risk of bladder tumor development. Several proteins have been selected as potential biomarker candidates for verification by immuno-based or MRM assays in additional individual specimens. Proteomic analysis of urinary microparticles reveals strong association of TACSTD2 with bladder cancer. TAGLN2 shows the most significant overexpression in bladder cancer tissues and might be a useful molecular tumor marker for evaluating bladder cancer lymph node metastasis. Urinary TAGLN2 and STMN1 also represent potential biomarkers for non-invasive screening of bladder cancer. Overall, the urinary concentrations of the classical plasma proteins or acute phase proteins show the best AUC values for discrimination between age-matched control and bladder cancer patients. The specificity of the acute phase proteins may be improved through the integration of tissue-leakage proteins as a biomarker panel.

Our findings highlight the value of integration of multiple clinical proteomes in providing valuable information for protein origin, specificity and application for future validation studies of potential biomarkers in bladder carcinoma.

### ML-08.02.5-001

#### Personalized therapy with metformin: effects of genetic variants of membrane transporters on type 2 diabetes-related traits

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Metformin is the first-line drug for treatment of Type 2 diabetes (T2D). Genetic variations of membrane transporters are implicated in the highly variable drug response. Here we analyzed association of several common variants, such as *SLC22A1/2* and *SLC47A1/2* genes encoding multidrug and toxin extrusion protein 1 (MATE1) and MATE2-K transporters, with broad spectra of phenotype data prior to and upon metformin treatment.

This study included 92 newly diagnosed, drug-naïve T2D patients. The genetic analyses were performed by Real Time-PCR. We collected phenotype data including, but not limited to, levels of fasting glucose (FG), insulin (FI), HbA<sub>1c</sub>, lipid levels, and anthropometric measures, prior to and 6 and 12 months post-metformin treatment.

Our data demonstrated significant association of *SLC47A1* rs2252281 variant with higher decrease of FI levels ( $P < 0.05$ ) and lower HOMA-IR ( $P < 0.05$ ) upon 6-month treatment. Furthermore, *SLC47A1* rs2252281 and *SLC22A1* rs622342 were associated with higher HDL-cholesterol ( $P = 0.027$  and  $P = 0.032$ , respectively), while other variants of these two genes (rs2289669/rs12208357) appear to increase LDL-cholesterol levels ( $P = 0.034$  and  $P = 0.001$ , respectively) upon metformin treatment. Interestingly, we showed that *SLC47A2* rs12943590 was associated with lower decrease of FI levels ( $P < 0.01$ ) and higher decrease of HOMA-IR ( $P < 0.01$ ) upon 6-month treatment in T2D patients, which is in line with the *in vitro* data showing that this variant increases transporter activity resulting in decreased drug effect.

Our results indicated that MATE1 variant carriers might have favorable effects on markers of insulin resistance and lipid status upon metformin treatment. Furthermore, other common variants of OCT1 and MATE1/2 transporters appeared to affect levels of these markers upon treatment, suggesting their important role in optimal response to metformin.

### ML-08.02.5-002

#### Genetic variability in *SLC5A2* influences glycaemic control and risk for diabetic retinopathy in type 2 diabetes patients

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Kidneys participate in glucose homeostasis by gluconeogenesis and renal glucose excretion. Up to 99% of the filtered glucose is reabsorbed in proximal tubules via sodium-glucose cotransporters (SGLTs). Mutations in SGLT2, coded by *SLC5A2* gene were found in familial renal glucosuria. We investigated if common *SLC5A2* rs9934336 polymorphism influences glucose homeostasis and risk for macro or microvascular complications in Slovenian type 2 diabetes (T2D) patients.

In total 181 clinically well characterised T2D patients were genotyped for *SLC5A2* rs9934336G>A polymorphism. Associations with glycaemic control and T2D complications were analysed with nonparametric tests and logistic regression.

Patients aged 64 (58.5–70.5) years had median duration of T2D 11 (6–17) years. They had relatively well controlled blood glucose, lipid levels and blood pressure on the prescribed treatment regimens. *SLC5A2* rs9934336 genotype distribution was as follows: GG 55.8%, GA 37.0% and AA 7.2%. Median (25%–75% range) basal blood glucose levels were 6.96 (6.40–7.99) mmol L<sup>-1</sup> in patients with GG, 7.96 (7.31–8.83) mmol L<sup>-1</sup> in GA and 9.04 (7.43–10.51) mmol L<sup>-1</sup> in AA genotype ( $P < 0.001$ ). The association of rs9934336 genotype with HbA1c levels was significant under the dominant genetic model ( $P = 0.030$ ). Retinopathy was the only late T2D complication associated with *SLC5A2* polymorphism after adjustment for T2D duration. Carriers of at least one polymorphic *SLC5A2* rs9934336 A allele had significantly higher risk for diabetic retinopathy than non-carriers (OR = 7.62; 95% CI = 1.65–35.28;  $P = 0.009$ ).

In conclusion, *SLC5A2* polymorphisms may influence the physiologic process of glucose reabsorption in kidneys in T2D patients. Furthermore, we report for the first time the association between *SLC5A2* polymorphism and diabetic retinopathy. Further studies are needed to investigate if genetic variability of SGLT2 transporters influences treatment outcome with the novel class of antidiabetic drugs that inhibit SGLT2.

## Thursday 8 September

9:00–11:00, Hall E

### Cardiac regeneration: Programming human heart cells

#### S-06.02.5-001

#### Isogenic human pluripotent stem cell pairs to study long-QT syndrome

M. Bellin

Leiden University Medical Center, Leiden, the Netherlands

Induced pluripotent stem cells (iPSCs) were first generated 10 years ago. Their ability to differentiate into any somatic cell type of the body including cardiomyocytes has already made them a valuable resource for modelling cardiac disease and for drug screening. Despite their immature phenotype, human iPSC-derived cardiomyocytes successfully recapitulated arrhythmic conditions including the long-QT syndrome (LQTS), which is associated with sudden cardiac death.

By precise gene targeting, we generated isogenic pairs of human patient-specific iPSCs for two representative forms of LQTS: the autosomal dominant LQTS type 2 and the autosomal recessive Jervell and Lange-Nielsen syndrome (JLNS). These cells were then differentiated into cardiomyocytes.

By eliminating the genetic background variations that can confound disease traits, we could quantify the specific reduction in the cardiac potassium repolarising currents I<sub>Kr</sub> (LQTS type 2) and I<sub>Ks</sub> (JLNS) and reveal the pathogenic molecular mechanisms underlying the disease phenotypes. In particular, we proved that in both cases the mutated channels had a trafficking defect. Furthermore, we tested a novel molecule acting as allosteric modulator of the hERG cardiac potassium channel that was able to rescue the LQTS phenotype.

Our study demonstrates that isogenic pairs of human pluripotent stem cells can be used to (1) prove the authenticity of the genotype–phenotype correlation; (2) unravel the pathophysiological mechanism seen in genetically inherited cardiac diseases; and (3) test drug sensitivity. As such, this approach represents a robust strategy to study not only cardiac disease mechanisms but also other genetic disorders.

### The effect of boron and boron minerals on preventing doxorubicin induced cardiotoxicity

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**Introduction:** Doxorubicin (DXR) is an effective chemotherapeutic agent causing severe cardiac failure over cumulative drug dose. Thus, preventing toxicity and using DXR over these doses can improve the success of oncologic treatment. Various agents especially antioxidants were used for preventing cardiotoxicity.

**Material and Methods:** The boron and mineral compounds tested were as follows: boric acid, borax, colemanite and ulexite. The study consisted of 78 Wistar-Albino male rats. The rats were randomly separated into 9 study groups and one control group. All study groups were administered with 1 mg DXR intraperitoneally. The groups except control group were administered with boric acid, borax, ulexite, colemanite and dexrazoxane. At the end of the study period, the homogenized tissues were analyzed using photometric, spectrophotometric or immunochemical assays.

**Results:** The median levels of serum MDA, SOD, GSH-Px, IL-1 $\beta$ , AST, LDH, CK, hs-TnT and NT-proBNP and significant difference from the control or sham groups. The NT-proBNP levels

in the study groups were not significantly different from those in the control group.

**Discussion:** Adriamycin is an effective drug in cancer treatment but cardiotoxicity is the limiting side effect. Although dexrazoxane is the leading drug in clinical use, various agents with low cost, more efficient and findable were evaluated to reduce the cardiotoxic side effects of the drug. This study showed that the borate minerals and dexrazoxane were effective to prevent DXR-cardiotoxicity.

**Conclusion:** In conclusion, we evaluated the cardioprotective effects of these boron compounds with their antioxidant, metal chelating and anti-inflammatory effects and found some minerals useful. Further studies with different dose schedules can be designed for increasing efficiency.

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