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DISSERTATION SUMMARIES

Development and characterization of different three-dimensional lung carcinoma cultures

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Screening of antitumor agents on human lung cancer cell lines has become essential in pharmaceutical research practice. Although, the most widely used technique is the two-dimensional monolayer cell culturing, it cannot adequately mimic the three-dimensional, solid, *in vivo* tumor microenvironment, hypoxia, growth and the rivalry for nutrients.

To solve these problems, and to determine more accurate responses to anti-tumor drug interventions novel, three-dimensional *in vitro* tumor models can give an option to better represent the complexity of *in vivo* tumors and their microenvironment.

The aim of our study was to develop and characterize different *in vitro* three-dimensional lung carcinoma cultures using A549 human adenocarcinoma cells. First, we measured the growing kinetics of differently cultured A549 cancer cells and we found differences between conventional monolayer cultured cells and three-dimensional cultured cells (A549 cells levitating with microcarriers, without microcarriers and RAFTTM islets). The cells grown three-dimensionally reached their stationery phase minimum two days later than conventional monolayer cultures, but their viability remained constant, it did not reduce under 80% in the exponential phase. We hypothesize, that the anaerobic conditions in case of the three-dimensional *in vitro* tumors are similar to the conditions of *in vivo* tumors, where the cells in the different layers are competing for nutrients and oxygen, resulting in activated expression levels of metabolic enzymes to provide a supply of energy. Conventionally cultured, monolayer cultures do not have selective pressure to activate their different metabolic and/or hypoxic enzymes. Therefore, we also measured the expression levels and found significant changes in MCT4 and GLUT1 mRNA expression between monolayer cultures and levitating three-dimensional microcarrier-free tumor spheroids. One of the explanations could be that expression induction of these genes could regulate the glycolytic switch of cancer cells, which might lead to reprogram tumor cells preparing for three-dimensional growth and altered metabolism.

Our approach for screening novel drug targets and chemotherapeutical agents in a three-dimensional in vitro lung carcinoma cellular model can more easily be translated into *in vivo* studies and later into clinical practice. By using these novel and not yet fully characterized *in vitro* three-dimensional models, we might also identify novel proteins that may be promising pharmacological targets for anti-cancer therapy in the future.

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Regulation of the nicotinic acid degradation pathway and study of the evolution of the pathway enzymes

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Many microorganisms can utilise nicotinic acid as a nitrogen source, even if the degradation pathway of nicotinic acid was studied only in a few prokaryotes. Because of the industrial potential of the pathway enzymes, the research topic of nicotinic acid degradation is popular in prokaryotes. Four different catabolic routes were described in these organisms, but according to our research the eukaryotic pathway differs from the prokaryotic routes.

We aimed to unravel the genetic and biochemical background of the nicotinic acid degradation in the eukaryote *Aspergillus nidulans*.

We have identified three genomic clusters, NDC1 (Nicotinic acid Degradation Cluster 1 - *hxnS, hxnR, hxnP, hxnT, hxnY, hxnZ*) and NDC2 (*hxnX, hxnV*) and NDC3 (*hxnN, hxnM*). All cluster genes are induced by nicotinic acid or 6-hydroxynicotinic acid metabolite and the pathway specific transcription factor, HxnR. HxnR is a member of NDC1 and encodes for a 852 amino acids long Zn(2)Cys(6) binuclear transcription factor, which occurs only in fungi. We obtained *hxnR* deletion mutant and together with $hxnR^{+}$ control and $hxnR^{c}$ constitutive mutants, the expression profile of the NDC cluster genes were studied. All cluster genes are expressed on both non-induced and induced conditions in the $hxnR^{c}$ mutant, while cluster genes are expressed only under the induced conditions in the $hxnR^{+}$ control strain. As expected, there is no gene expression of the cluster genes in the *hxnR*Δ strain. We have isolated thirty constitutive mutants after UV mutagenesis on selective media, which produced constitutively the purine hydroxylase II enzyme, HxnS, as we showed by enzyme activity assays. In the

thirty constitutive mutants we have identified 17 different mutations by sequence analyses. We detected putative HxnR orthologues only among the Pezizomycotina and we compared 23 orthologs and constructed a CONSURF profile of the protein. All constitutive mutations mapped in highly conserved regions, in two patches. A well defined patch between residues 219 and 239, in a wider domain between residues 565 and 639, located near to the carboxy terminal. For a number of residues we have obtained several different amino acid changes. We could propose, that aromatic residues at position 226, 228 and a basic residue at position 605 are necessary to maintain the protein in its physiological inactive state.

We investigated intracellular localization of HxnR under non-induced, induced and repressed condition by the study of a HxnR-Gfp expressing strain, where expression of *hxnR-gfp* was driven by the proline induced *prnD* promoter. HxnR-Gfp shows nuclear localization under all conditions.HxnS catalyses the hydroxylation of nicotinic acid to 6-hydroxynicotinic acid and also hypoxanthine to xanthine. We proposed that HxnS originated from the duplication of the ancestor of the recent purine hydroxylase I coding gene (*hxA*), which is regulated by the purine utilization pathway specific UaY transcription factor, belongs to the purine utilization pathway and the gene product hydroxylates hypoxanthine to xanthine and further hydroxylates xanthine to uric acid. We conducted an extensive comparative structural-and-functional study of HxnS and HxA, which resulted in the presumptive identification of those amino acid residues, which endow HxnS with the nicotinic acid hydroxylating properties. These results made possible to design *in vitro* enzyme evolution studies.

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Stress tolerance in plants: the role of heat shock factors and MAP kinases

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Heat shock factors (HSFs) mediate the activation of large set of stress-induced genes upon exposure to high temperature or other stress conditions by trimerization and subsequent binding to the conserved heat shock elements (HSE) of the promoter regions of the targets. Earlier we have identified the heat shock factor A4A (HSFA4A) of *Arabidopsis thaliana*, which confers salt and oxidative stress tolerance to overexpressing plants. HSFA4A was found to display dimerization in plant cells and this phosphorylated by mitogen-activated protein kinases MPK3 and MPK6 on Ser309, which is required for its activation (Pérez-Salamó et al. 2014).

While phosphorylation of HSFA4A was clearly demonstrated by MPK3/6 kinases, MPK4 failed to phosphorylate this transcription factor, suggesting that only the MPK3/6 cascade is responsible for its activation. Site-specific mutagenesis was used to generate Ser309Ala and Ser309Asp mutants, to abolish or mimic phosphorylation of HSFA4A, respectively. Bimolecular Fluorescence Complementation (BiFC) assay was used to compare dimerization of mutated and wild type HSFA4A proteins in plant cells. While dimerization of the Ser309Asp mutant was comparable or stronger than wild type HSFA4A, Ser309Ala mutation abolished homomeric interactions, suggesting that phosphorylation is important for the active trimer formation of this heat shock factor. Preliminary results showed, that Arabidopsis plants overexpressing the Ser309Asp phosphomimic version of HSFA4A have superior salt tolerance when compared to transgenic lines overexpressing the wild type factors. These results suggest that MAP kinase-mediated phosphorylation is essential for intramolecular interactions of HSFA4A and important for the defense-related function of this heat shock factor.

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The role of plant glutathione peroxidases

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Environmental factors affect development, growth and productivity of plants throughout their lives. If the values of different environmental factors differ from the optimum – *e.g.*, too low or high temperatures, water deficiency – stress occur. Plants are forced to defend themselves against detrimental effects and adapt to suboptimal living conditions, modulate their metabolism and expression pattern of genes and proteins. Antioxidants are important elements of the defense against stresses, among them, the glutathione peroxidases (GPXs), which are

common enzymes, found in animals, fungi and plants. These proteins scavange the reactive oxygen species, catalyze reduction of hydrogen peroxide (H_2O_2), organic hydroperoxides and lipid peroxides using glutathione or other reducing components, such as thioredoxin. The plant glutathione peroxidases are mostly similar to animal phospholipid hydroperoxide glutathione peroxidases (PHGPX). These PHGPXs play a very important role in protecting against oxidative damage of membranes. In addition to the possible antioxidant functions, plant GPXs also participate in redox signaling and plant development. The *Arabidopsis thaliana* contains 8 glutathione peroxidase isoenzymes, however, their role in plant development and stress responses and their exact mechanisms are not well-known.

Our aim was to characterize the Arabidopsis GPX enzymes. The substrate specificity and other enzymatic properties were investigated using recombinant proteins, produced and purified from *E. coli*. Other experiments were performed on T-DNA insertion mutants (SALK_128885C; SALK_082445C; SALK_071176C; SAIL_623_F09; SALK_076628C; WiscDsLox321H10; SALK_072007C; SALK_127691C) in different growing and treatment conditions. We determined the *in vitro* malondialdehyde and H_2O_2 contents, non-enzymatic antioxidant ascorbate and glutathione contents, activity of antioxidant enzymes: glutathione reductase, glutathione transferase, glutathione peroxidase, thioredoxin peroxidase, guaiacol peroxidase, catalase, superoxide dismutase. We compared the root length and germination rate of the different T-DNA insertion mutants during osmotic stress.

Based on our results, affinity of glutathione peroxidases differ to the lipidperoxides and H_2O_2, and it also depends on the specific thioredoxin cosubstrates. In the mutant plants not only the activity of enzymatic antioxidants (glutathione peroxidase, thioredoxin peroxidase, glutathione transferase) changed, but the level and reduction state of non-enzymatic antioxidants, such as glutathione and ascorbate too. The drought or osmotic stress experiments conducted on *Atgpx1-8* mutants demonstrated, that the AtGPX3 and 5 have important roles in the development, while the AtGPX2, 4, 6 and 8 especially in the stress responses.

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Sumoylation of phytochrome interacting factor 3 alters photomorphogenesis in *Arabidopsis*

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Besides light, there is a crucial abiotic factor for plants providing energy for photosynthesis, it is also an important environmental signal what plants constantly monitor to increase their fitness and survival rate. Light perception leads to the induction of light-grown developmental program, called photomorphogenesis. Plants capture photons with different photoreceptors, each responsible for monitoring certain light wavelengths. There are five phytochromes (phy, named phyA-E) in the widely used model plant *Arabidopsis thaliana,* which is the sensor of red and far-red radiation (c. 600-730 nm). Among them, phyB is the dominant receptor of red light-induced responses. PhyB can initiate complex signaling pathways, resulting in modified expression of about 3000 genes leading to changes in the developmental programs of plants. Posttranslational modifications (*e.g.*, phosphorylation or SUMOylation) of the proteins of these pathways provide fine tuning possibility of the regulatory system. During SUMOylation, a SUMO (Small Ubiquitin-like Modifier) protein binds to a certain amino acid residue and will be able to modify the function of the target protein. Recently it was shown, that both phosphorylation and SUMOylation of phyB photoreceptor negatively regulates its signaling via different molecular mechanisms.

Our knowledge about the posttranslational modification of the elements of phyB controlled pathways is rather limited. We performed an *in silico* modeling analysis, which revealed that a lysine residue of the Phytochrome Interacting Factor 3 (PIF3) is a target of SUMOylation. Furthermore, we found that PIF3 is SUMOylated *in vitro* using a bacterial system. PIF3 is a basic Helix-Loop-Helix (bHLH) type transcription factor, which interacts directly with the physiologically active form of phyB and negatively regulates the phyB governed signaling. This interaction is necessary for the light-induced degradation of PIF3 protein, inducing pathways contributing to proper photomorphogenic development. *pif3* mutant seedlings show enhanced inhibition of hypocotyl elongation, whereas PIF3 overexpressor plants have long hypocotyls when they grow in red light.

To analyse the physiological effects of PIF3 SUMOylation we fused PIF3 and the mutant version of PIF3, where the SUMO-target lysine is exchanged to arginine to the Yellow Fluorescent Protein (YFP) and expressed the chimeric proteins in *pif3* null-mutant background. Phenotypic analysis of these transgenic plant lines revealed that the non-SUMOylated mutant PIF3 induces less pronounced inhibition of

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hypocotyl elongation. This finding indicates, that the removal of the SUMO resulted more active PIF3 molecules thus hyposensitivity of the phyB-controlled photomorphogenesis.

We found that PIF3 is SUMOylated and the mutation of the conserved lysine residue drastically reduces the SUMOylation of the PIF3 pool *in planta.* Furthermore, the extent of PIF3 SUMOylation is changing during light/dark phases, indicating that it is a dynamic process involving SUMO ligase and protease enzymes. We assume that the mechanistic explanation of the observed phenotypes is that SUMOylation can alter the binding of PIF3 and phyB molecules, and the modified balance of this interaction leads to the observed phenotypes. Next we plan to examine this interaction in our established *in vitro* and *in vivo* experimental systems.

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Investigation of the nicotinic acid degradation pathway in *Aspergillus nidulans*

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ln nature nicotinic acid (precursor of the vital NAD^+) can be synthesized endogenously or taken up from the environment by transporters. Many microorganisms possess the capacity to utilize nicotinic acid as sole N-source, however, the degradation of nicotinic acid was studied in only a limited number of prokaryotes that showed a surprisingly broad spectra of possible degradation pathways. In eukaryotes the nicotinic acid catabolic process is completely unknown, only the nicotinic acid hydroxylase (HxnS) of *Aspergillus nidulans* had been thoroughly studied. It catalyses the first step of nicotinic acid catabolism and has unprecedented substrate specificity. It accepts nicotinic acid as well as hypoxanthine as substrate and catalyses the hydroxylation of nicotinic acid to 6-hydroxynicotinic acid and also hypoxanthine to xanthine.

As a result of the research in our laboratory we identified 3 gene clusters, in which the genes are regulated by a common transcription factor (HxnR), therefore these genes have been associated with nicotinic acid degradation. The gene of the pathway-specific transcription factor (*hxnR*), the *hxnS*, *hxnP*, *hxnZ*, *hxnY* and *hxnT* belong to the first cluster (NDC1 - nicotinic acid degradation cluster 1), the *hxnX, hxnV* and *hxnW* genes belong to the second cluster (NDC2), and the *hxnN* and *hxnM* belong to the third cluster (NDC3). The study about the regulation of the pathway revealed, that all of the NDC genes are positively regulated by the HxnR transcription factor, and the general co-regulator AreA. The metabolic inducer of the pathway is a metabolite downstream to nicotinic acid. We deleted the genes of the clusters, and analysed the phenotypes of the deletion mutants. As a result, *hxnX, hxnV, hxnW,* and *hxnM* gene products were proved as members of the nicotinic acid catabolic route. HxnX, HxnW and HxnV are oxidoreductases, HxnM is a cyclic C-N hydrolase, HxnN is an amidase. HxnP and HxnZ are transmembrane domain proteins, putative transporters. HxnT is a NADH dehydrogenase, and HxnY is a Fe(II)-dependent dioxygenase. Both *hxnT* and *hxnY* deletion mutants utilize nicotinic acid as sole N-source, but show reduced growth on 6-hydroxynicotinic acid, which is the downstream metabolite of nicotinic acid. This finding indicates that an alternative route operates in the pathway downstream to nicotinic acid. The growth reduction of *hxnY* and *hxnT* double mutant is additive.

Since the order of these genes is not revealed yet, we aim to shed light of the sequential order of the enzymes by growth tests and GC-MS analysis. By *in silico* analysis we examined the cellular localization signals of the cluster genes and we started to generate carboxy- or amino terminal fused GFP-fusion protein expressing strains to study the intracellular localization of the pathway enzymes and transporters. We expect a peroxisomal localization of HxnX and membrane (cytoplasmic or vacuolar membrane) localization of HxnP and HxnZ. The rest of the enzymes do not carry characteristic localization signals, thus their cytoplasmic localization is expected.

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Transcriptome profiling of TNBS induced rat model of IBD

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Inflammatory Bowel Disease (IBD) is a chronic relapsing disorder of the gastrointestinal tract. IBD comprises two main type of intestinal inflammation: Crohn's disease (CD) and ulcerative colitis (UC). These are characterized by chronic inflammation along the digestive tract. IBD is a multifactorial disorder, it is associated with genetic and environmental factors in combination with lifestyle. Although, genome-wide association studies revealed susceptibility loci in IBD risk, genetic heterogeneity was demonstrated between trans-ancestry populations and its etiology is still unknown. Understanding of the background mechanism of IBD is important, because its incidence is increasing worldwide.

Animal models of IBD have been developed to study the pathogenesis of disease and potential therapeutic targets. There are various types of experimental murine models of colitis from chemically induced to genetically manipulated.

In the case of 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced rat model of IBD, TNBS is intracolonically administered. After treatment, the size of lesions in colonic mucosa are variant among treated animals, even so, sample collection is achieved lengthwise from the colon in most cases. Ulceration, loss of epithelial cells and immune cell infiltration will be distinct between inflamed and uninflamed regions of the colon, which has a great impact on gene expression levels.

Whole transcriptome analysis is a powerful tool to identify disease related genes and observe changes in signaling pathways. To demonstrate transcriptional changes after TNBS treatment in different regions of the colon, RNA-Seq was applied and transcriptomic data was interpreted by Ingenuity Pathway Analysis (IPA).

Multidimensional scaling (MDS) of individual transcriptomic data clustered samples into three groups. Control, TNBS treated uninflamed and TNBS treated inflamed samples markedly separated from each other. At gene expression level, TNBS treated uninflamed rat colon regions are more similar to the control samples than to the TNBS treated inflamed colon sections suggesting that individual variations in longitudinally collected TNBS treated rat colons, the current "gold standard", may lead to false interpretation of the results.

Functional analysis of our data set revealed several previously known canonical pathway, related to inflammation or immune response were highly activated in the inflamed regions, *e.g.*, acute phase response, leukocyte extravasation, pattern recognition receptor and Il-6 signaling, with a correlation of enhanced expression of proinflammatory cytokines and chemokines (*e.g.*, Tnf, Il-6, Il-10, Ccl3 and Cxcl1). On the other hand, in TNBS treated uninflamed samples, these pathways and molecules were not induced; in contrast, the expression of glycosyltransferases (GTFs), fucosyltransferases (FucTs) and mucins significantly increased. GTFs and FucTs are involved in the synthesis of carbohydrate antigens with functions in immune cell recruitment and protection of endothelial cells, and in the case of Fut4, in cell proliferation. Additionally, they are responsible for the glycosylation of Mucin2, which arranges the two mucous layer of colon, protects epithelial cells against hazardous agents and serves as connection sites to commensal flora. Interestingly, its altered glycan profile was observed in UC patients. These findings highlight the importance of the investigation of the uninvolved regions of the colon after TNBS.

These results indicate the importance of the modified sample collection in TNBS induced rat model of IBD. Furthermore, the investigation of the uninflamed regions of TNBS treated animals has the potential to find novel molecules involved in IBD pathogenesis.

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The role of cell wall integrity of *Candida parapsilosis* during host-pathogen interaction

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Candida species are opportunistic fungal pathogens, causing serious infections in immunocompromised patients. Besides *C. albicans*, which is the most common species responsible for invasive candidiasis, the incidence of "non-albicans" species, such as *C. parapsilosis* has largely increased over the past decades. The pattern recognition receptors (PRRs) of the innate immune system are the first line of defence against infection, being able to recognise and initiate an inflammatory response to invading microorganisms. The cell wall is the immediate point of contact between these pathogens and host, influencing the antigenicity and the modulation of the immune response.

During our study, we examined the role of cell wall integrity in the pathogenicity and immune sensing of *C. parapsilosis*. The effects of *N*-linked mannosylation were investigated in the *och1*Δ/Δ *C. parapsilosis* (*Cpoch1*Δ/Δ) strain, which lacks an -1,6-mannosyltransferase that is responsible for the construction of the *N*-mannan outer chain in the cell wall. In a mouse model of systemic candidiasis we compared the susceptibility of wild-type BALB/c mice to *C. parapsilosis* wild-type and *Cpoch1*Δ/Δ strain infection. The *Cpoch1*Δ/Δ null mutant was significantly attenuated in virulence with decreased fungal burdens in the spleen, kidneys and liver at 1, 3 and 7 days post-infection compared to the wild-type strain. To explore the mechanism of the decreased virulence of the *Cpoch1*Δ/Δ strain, we investigated the function of Dectin-1 receptor (one of the most intensively studied C-type lectin) in the recognition of *C. parapsilosis* in *in vivo* experiments. After intravenous infection with *C. parapsilosis* wild-type and *Cpoch1*Δ/Δ strain, we evaluated the fungal burdens in C57BL/6 wild-type and Dectin-1 receptor mutant mice. Infection with *Cpoch1*Δ/Δ mutant strain led to different fungal loads in various organs (spleen, kidney, liver and brain) between the C57BL/6 wild-type and Dectin-1$^{-/-}$ mice; *Cpoch1*Δ/Δ-challenged Dectin-1$^{-/-}$ mice showed higher susceptibility to infection compared to C57BL/6 wild-type mice. Nevertheless, infection with the *C. parapsilosis* wild-type strain resulted in similar fungal burden in C57BL/6 wild-type and Dectin-1$^{-/-}$ mice. In this murine model, we also examined the recruitment of different immune cell populations to infected organs. Examination of blood samples showed a strong drop of lymphocyte numbers and increased percentage of neutophil population of *Cpoch1*Δ/Δ-challenged Dectin-1$^{-/-}$ mice in comparison to C57BL/6 wild type mice infected with either wild type or *och1*Δ/Δ *C. parapsilosis* at 1 and 3 days post-inoculation. Using flow cytometric analysis we identified the different immune cell subsets of spleen, kidney and liver of wild type and Dectin-1$^{-/-}$ mice challenged with both *C. parapsilosis* wild-type and *Cpoch1*Δ/Δ. Our results indicated an early, increased influx of neutrophils and higher recruitment of macrophages in spleen, liver and especially in kidney of Dectin-1$^{-/-}$ mice after *Cpoch1*Δ/Δ infection compared the other groups of mice. Furthermore, we also observed that lack of Dectin-1 receptor in mice resulted in a decreased ability to recruit natural killer cells during *C. parapsilosis* wild-type infection. Taken together, our results demonstrate that *N*-linked mannosylation significantly affects the virulence and the specific steps of recognition of *C. parapsilosis in vivo*. Furthermore, our findings contribute to a detailed comprehension of the immune modulating role of *C. parapsilosis* cell wall structure, which will yield deeper insights into host-pathogen interactions during *C. parapsilosis* infection.

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Biotechnological application of bacteriophages

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Mutidrug resistant pathogenic bacteria are rapidly emerging and spreading. They might cause untreatable human diseases, serious economic loss in animal husbandry and agriculture. Therefore, new alternative therapeutic approaches are necessary. One possibility is to use bacteriophages, the natural enemies of bacteria. Bacteriophages or shortly phages are the obligate intracellular parasitic viruses of bacteria. They can specifically infect bacterial cells and have three kinds of life cycle strategies: a) productive infection with cell lysis, b) productive infection without cell lysis, c) integration into the host DNA. For bacteriophage therapy, phages belonging to the group a) can be used. There are numerous advantages of phage therapy including specificity, costs, minimal side effect, various applicability, propagation at the site of infection, combined applicability etc..

In our laboratory, we studied phages and phage proteins against numerous plant, animal and human pathogens including *Erwinia*, *Xanthomonas*, *Salmonella*, *Bortadella*, *Listeria* and *Staphylococcus* phages.

Out of these, the genomes of numerous phages have been sequenced involving phages against the plant pathogen *Xanthomonas arboricola* pathovar *juglandis* infecting walnut. It causes walnut blight and can reduce the yield by more than 50%. Multiple lytic phages have been isolated against this pathogen. They could be successfully applied against the pathogen *X. arboricola* pathovar *juglandis* in laboratory. Their genomes have been sequenced and these genomes are the first sequenced ones from phages that are able to infect *Xanthomonas arboricola* pathovar *juglandis*. After the genome sequencing, the proteins have been annotated and genome scale sequence comparison was performed. Among phages of animal and human pathogens, several genomes of both temperate and lytic phages of *Bortadella*, *Staphyloccus* species have been sequenced and compared. In few cases, the experimental structural protein profiles of the phages were also compared to the deduced proteins.

Enzybiotics – in general – are natural enzymes having antibacterial or antifungal activity. Originally, this term was used for the phage enzymes, such as endolysins, capable to degrade bacterial cell wall. There are endolysins of various activities cleaving the peptidoglycan at distinct positions.

Endolysins produced by bacteriophages and they are also good candidates against bacteria since -similarly to phages – these enzymes

are host specific, as well. They are synthesized during the late stage of infection and also responsible for destroying the bacterial cell wall, which eventually leads to the lysis of the host cell and liberation of the newly formed progeny virions. Since Gram+ bacteria have no outer membrane and their cell wall is readily accessible from the outside, so these enzymes can be used against them.

Our goal is to produce a mix of endolysins capable to kill the target *Listeria monocytogenes* strains. Four candidate enzymes have been chosen from different *Listeria* bacteriophages and heterologous expression systems are being developed to express them for basic research and industrial applications.

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The cell-mediated immunity of the honey bee (*Apis mellifera*)

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The honey bee (*Apis mellifera*) is not just vital in producing bee products, which are used in food industry, pharmaceutics and cosmetics, but it is also very important in maintaining biodiversity. Recently the spread of Colony Collapse Disorder (CCD) caused major economic and ecologic losses, supposedly by homeostatic imbalance. The CCD is a multifactorial phenomenon generated by the combination of biotic- (the wide range of honey bee diseases) and non-biotic factors (the increasing use of pesticides in the environment). In order to find a solution for the CCD caused damage, it is crucial to understand the machinery of the honey bee immunity, considerable efforts and new strategies have to be developed and applied to improve the bees' fitness. The honey bee is a social insect it has communal defence mechanisms, such as hygienic behavior and hive fever. The individual immune system consists of regulatory pathways of the humoral immunity and the cell-mediated responses. The effector cells of cellular immunity are the blood cells, so called hemocytes, which engulf microorganisms, produce antimicrobial peptides and form capsules around intruders, which are too big to be taken up by phagocytosis. Recently considerable efforts are being devoted in different laboratories around the World to characterize the cellular components, however, due to the lack of molecular markers for these cells the results are controversial. So far the haemocytes were characterized based on their morphologic and lectin binding properties, but more specific method would be necessary to study the function of the different types, to follow the differentiation of the blood cells and to reveal the hematopoesis of the honey bee.

We developed a toolkit for the characterization and classification of the honeybee hemocytes by cell-type specific antigens. In our experiments '*in vivo*' and '*in vitro*' immunological and molecular techniques were used. With the combination of a defined marker panel and functional tests, we have classified the hemocyte subsets of the honey bee. The markers distinguish the melanizing oenocytoids, the phagocytic granulocytes and the encapsulating plasmatocytes. According to Western-blot, MALDI analysis and RNA interference the plasmatocyte specific marker was identified as the honey bee hemolectin. It has substantial homologies with the human von Willebrand factor. It is produced by the plasmatocytes forming a fibre matrix of the coagulum after wounding by parasites and other mechanical agents.

With the haemocyte marker, panel analytical studies can be carried out to study the cell mediated immunity of the honey bee and it is a possible tool for the identification of haematopoietic tissues and haemocyte lineages. Our studies expand the knowledge of the honey bee cell mediated immunity, and we anticipate that they will be helpful in understanding immune defence against parasites, microbes and agents in the honey bee, including CCD.

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Investigation of inoculation methodology and the genetic background of *Fusarium* head blight (FHB) resistance in wheat

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Fusarium head blight (FHB) is an important disease of wheat (*Triticum aestivum* L.), causing not only yield losses, but also mycotoxin contamination of kernels. It is known that flowering is the most favorable host developmental stage for *Fusarium* infection in wheat. Sus-

ceptibility window (SW) defines the period of receptivity, while *Fusarium* species can cause serve infection during and after anthesis. The length of this period is an important factor in practical aspects too, because it can influence the application of fungicide treatment and the designation of artificial inoculation times. In *Fusarium,* resistance studies are particularly important factors, because most of these studies work with experimental populations consisting wheat genotypes with different resistance level and flowering time. In previous studies, the length of the most susceptible period was specified from 7 to 10 days after anthesis, but it is not clarified if it is influenced by the resistance level of the host. This study investigated the SW to *Fusarium* head blight in 7 winter wheat genotypes differing in resistance level. The plant material was evaluated in 2 experimental years (2013-2014), inoculated with 2 *Fusarium culmorum* and 2 *Fusarium. graminearum* isolates in six inoculation dates: mid-anthesis then 4, 7, 10, 13 and 16 days after mid-anthesis. Disease levels were estimated by visual symptoms of FHB, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content.

The phenotypic variation of many complex traits (*e.g.*, *Fusarium* head blight resistance) is influenced by multiple quantitative trait loci (QTLs). Linkage analysis and association mapping (AM) are the two most commonly used tools for analyzing complex traits. AM involves searching for genotype-phenotype correlations among unrelated individuals. Conventional linkage analysis with experimental populations derived from a bi-parental cross provides information about traits that tends to be specific to the same or genetically related populations, while results from association mapping are more adaptable to a wider germplasm base.

In our study, 95 hexaploid wheat varieties and lines of mainly European origin were evaluated for resistance to *Fusarium* head blight in 2015. Disease levels were estimated by visual symptoms of FHB and FDK. The experimental population was genotyped using Kompetitive Allele Specific PCR (KASP) technology. In total, 860 of 960 tested SNP markers were polymorphic and could be used for further analysis. With use of the mapped polymorphic marker data and the phenotype data genome-wide association mapping were performed using mixed linear model (MLM) statistics to identify marker-trait associations. Marker associations with *Fusarium* head blight resistance were detected on chromosomes 1B and 5B, based on visual FHB symptoms.

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Blood-brain barrier changes in acute pancreatitis: testing protective agents in cell cultures and animal experiments

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The early steps in the pathogenesis of severe acute pancreatitis, an inflammatory disease,which is not known in details. Pancreatic encephalopathy characterised by brain oedema and neuronal death develops in one fifth of patients with acute necrotising pancreatitis. The symptoms of this complication raise the possibility of blood-brain barrier (BBB) injury. Our group previously demonstrated elevated BBB permeability and increased blood cytokine levels such as interleukin-6 and tumor necrosis factor-α (TNF) in taurocholate induced acute pancreatitis in rats (Farkas et al.1998). A new non-invasive animal model has been recently developed and characterised in which acute pancreatitis is induced by the intraperitoneal injection of cationic amino acids (L-ornithine, L-lysine, L-arginine; Biczó et al. 2011). Our goal was to examine blood vessel permeability in this new pancreatitis model in different organs (3 g/kg bw ornithine i.p.) and further examine the mechanism of endothelial barrier injury. We also wanted to find new protective molecules for brain endothelial dysfunction. We demonstrated in L-ornithine induced acute pancreatitis tissue sensitivity and morphological changes in the vessels of pancreas and brain, but not in liver and lung. Expression level of the main cationic amino acid transporter, Slc7a1 was temporarily increased in the sensitive tissues during the development of pancreatitis. The direct effect of L-ornithine was also studied on a culture model of the BBB, primary rat brain endothelial cells co-cultured with pericytes and glial cells. The permeability of this *in vitro* BBB model was elevated by L-ornithine (20 mM), but not by D-ornithine. Ornithine treatment also led to damage of brain endothelial glycocalyx, an important element of the barrier. Elevated levels of nitric oxide and reactive oxygene species, as well drastic changes in the mitochondrial network were seen in ornithine-treated endothelial cells. The cationic amino acid treatment caused similar changes in the gene expression level of Slc7a1 as found *in vivo*.

Since brain capillary endothelial cells are damaged in several pathologies, including systemic pathologies, like acute pancreatitis, protection of the BBB emerges as a new therapeutic target in recent years. Proinflammatory cytokines TNF and interleukin-1β participate in both central and peripheral inflammations, and were chosen to model BBB damage. Two molecules with proven anti-inflammatory effects, which were not examined for their effects on the BBB were selected for our next study. A neuropeptide, α-melanocyte stimulating hormone (MSH) was protective in ischemia-reperfusion and inflammatory studies in animal models. The other compound, SZV-1287, an inhibitor of vascular adhesion protein-1 also prevented the effects of inflammation *in vivo*. We found that MSH had a protective effect in low concentrations, against cytokine-induced damage in rat brain endothelial cells. The increase in permeability and reactive oxygen spe-

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cies production could be attenuated by MSH treatment. The nuclear localization of the inflammatory transcription factor NF- B was also decreased in MSH-treated cells. According to our preliminary data SZV-1287 could decrease the damage caused by L-ornithine in brain endothelial cells indicating a protective effect. In conclusion, we could demonstrate BBB damage in a new pancreatitis model, and found possible processes contributing to the pathomechanism. We identified the protective effect of MSH and SZV-1287 on brain endothelial cells in inflammatory conditions.

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Anatomical and functional characterization of the kynurenine system in the mouse brain

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Tryptophan is catabolized mostly through the kynurenine pathway (KP) in the mammalian brain. Kynurenic acid (KYNA) is a neuroactive end-product of this metabolic route. Altered brain KYNA level is implicated in several neurodegenerative and neuropsychiatric disorders such as Alzheimer's disease or schizophrenia. Therefore, manipulating KYNA synthesis or other branches of the KP with the aim of therapy holds broad therapeutic perspectives.

KYNA is produced from its precursor L-kynurenine (L-KYN) with the aid of kynurenine aminotransferases (KATs). From the four identified isoforms of KAT, KAT-2 has got the greatest importance both in the rat and human brain. Based on the anatomical studies performed in rats, the prevailing view is that KAT-2 is localized mostly in astrocytes in the brain. However, it has been reported that KAT-2 is presented also in rat and human neuronal cell cultures, and sporadically in neurons of the striatum and hippocampus of the rat brain.

The mechanism of KYN-KYNA transition and the role of KAT-2 were investigated in rat *in vivo* studies recently. In these experiments *de novo* KYNA synthesis was induced with the aid of L-KYN administration. Although, investigating the role of KYNA in neurodegenerative and neuro-psychiatric diseases in mice is of great relevance, neither anatomical nor functional studies have been performed alongside these pharmacological studies in mice.

The first aim of this study was to clarify the presence of KAT-2 mRNA and protein in adult mouse brain tissue. We examined the expression of kat-2 mRNA with *in situ* hybridization and performed fluorescent immunohistochemistry, to identify KAT-2 containing cells in brain structures in which the role of KYNA related to neuro-psychiatric disorders has been proposed formerly (hippocampus, striatum, substantia nigra and prefrontal cortex). The antibody used against KAT-2 was validated on mouse brain tissue homogenate and also on HeLa cells transfected with mouse KAT-2 cDNA.

The second aim was to induce *de novo* synthesis of KYNA in mouse brain slices *in vitro* for further electrophysiological and biochemical experiments related to KYNA and KAT-2 function. So our first question was, whether KYNA production increases upon exposure to L-KYN in our experimental protocol. We measured KYNA concentration of the incubation medium of control and L-KYN (10 μM) treated slices with high-performance liquid chromatography (HPLC).We detected the presence of KATII mRNA and protein throughout the adult mouse brain. In addition to the former expression data from the rat, we found prominent KATII expression not only in the astrocyte, but also in neurons in several brain regions (*e.g*., hippocampus, prefrontal cortex). Interestingly, in all brain areas the vast majority of the KAT-2 containing neurons were $GAD67^+$ interneuron.

Moreover, we demonstrated that there is a significant increase of KYNA level in the incubation medium of mouse brain slices after L-KYN administration. KYNA concentration in the L-KYN treated group was more than 100 fold higher than in the control group.

Our new finding related to the expression profile of KAT-2 attributes a new role to interneuron-derived KYNA in neuronal network operation. We also proved that incubation of mouse brain slices in L-KYN is a suitable way to induce *de novo* KYNA synthesis and provides appropriate basic for further studies of KAT-2 function in mice.

Furthermore, our results suggest that the thorough investigation of the spatio-temporal expression pattern of the relevant enzymes of the KP is a prerequisite for developing and understanding the pharmacological and transgenic murine models of kynurenergic manipulation.

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Investigation of the chemotherapeutic potential of metal-based nanoparticles in different cancer models

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Although, cancerous diseases lead the mortality rates worldwide, the standard therapy for overcoming tumors is still based on the cytotoxic effects of different chemotherapeutic drugs. In clinical cancer treatment, combinations of toxic compounds are systemically administered, however, their success rate strongly depends on the individual genetic nature of the given patient. Nano-sized materials are considered as the most potent novel candidates in pharmaceutical developments, due to their unique physical-chemical properties and favorable behavior in biological media. Metal-based nanoparticles excel among nano-biomaterials, since their biocompatibility, surface-plasmon resonance, simple synthesis and easy surface chemistry render them suitable tools for drug delivery, imaging and for tumor targeting as well. Silver and gold are the most commonly engineered noble metals in nanoparticle-based drug design. While silver nanoparticles are well known for their unique anti-microbial features, their anti-cancer activity has only been demonstrated recently. On the other hand, the fairly unreactive nature and inducible catalytic activities of gold nanoparticles are also valued in nanotechnology-based approaches, as these nanomaterials are frequently designed to function as delivery platforms to carry anti-cancer drugs or as unique radiosensitizers upon radiotherapy.

Despite the fact that these nanoparticles are attractive tools for future cancer medicine and have numerous advantages, the exact molecular mechanisms behind their activity in different cancers is still not well-described. Hence, we aimed to investigate the cellular and molecular effects of silver, gold and silver-gold hybrid nanoparticles in various *in vitro* and *in vivo* cancer models.

Inactivation of the p53 tumor suppressor is frequently observed in human cancers. Moreover, its loss-of-function mutation hinders the efficiency of chemotherapy-induced cancer cell apoptosis. Therefore, we studied the response of osteosarcoma cells with different p53 genetic backgrounds on silver nanoparticle administration and investigated the silver nanoparticle-provoked apoptotic mechanisms. The repeated administration of chemotherapeutic drugs often leads to the development of multidrug resistant cancer. Hence, we aimed to compare the silver nanoparticle-triggered apoptotic responses in drug sensitive and multidrug resistant colon adenocarcinoma cells as well. Finally, the anti-metastatic capability of gold-silver hybrid nanoparticles was also tested in Balb/c mice carrying orthotopic 4T1 breast tumors.

We found that both 35 nm and 5 nm diameter sized silver nanoparticles are taken up by cancer cells and induce apoptosis in a p53-independent manner. Our results suggest, that silver nanoparticles induce cancer cell death via stimulation of the mitochondrial apoptotic pathway. We also found that 35 nm sized silver nanoparticles hindered the growth of both drug sensitive and drug resistant cancer cells, and that the efflux activity of the multidrug resistant cancer cells can be inhibited by silver nanoparticle administrations. As silver nanoparticles synergistically enhanced the anti-cancer effect of six structurally and functionally unrelated chemotherapeutic agents in multidrug resistant tumor cells, we concluded that silver nanoparticles could be efficient combinational partners of conventional anti-cancer agents. Finally, we found that gold-silver hybrid nanoparticles were able to suppress the metastatic capability of 4T1 cells both *in vitro* and *in vivo*.

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Analysis of the *Drosophila* spectraplakin function during epithelial closure processes

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Dorsal closure of the *Drosophila* embryonic epithelium provides an excellent model system for *in vivo* analysis of molecular mechanisms regulating cytoskeletal rearrangements. Dorsal closure represents the last major morphogenetic movement during embryogenesis, when two opposed epithelial sheets converge toward the midline where they meet, sealing a hole at the dorsal surface of the embryo. The fusion of the epithelial sheets takes place by a zipper-like mechanism, as the opposing surfaces are zipped together at the most anterior and posterior ends of the dorsal hole. Genetic screening, biochemical and cell biological approaches have uncovered some of the structural and signaling molecules required for these closure events. Several studies have highlighted the importance of reorganization of actin-based structures, such as filopodia and lamellipodia, but the function of the microtubule (MT) network is very poorly understood.

To uncover novel components required for microtubule reorganization and function, we have applied an RNAi-based screening method combined with automated *in vivo* video microscopy and we identified the *short stop (shot)* to be essential for the zippering of the dorsal

hole. Shot belongs to the conserved family of the spectraplakins, gigantic structural proteins with functional domains binding to actin filaments, microtubules and cell adhesion complexes. Using isoform-specific mutant alleles and genetic rescue experiments with truncated Shot variants, we demonstrated that Shot functions, as an actin-microtubule cross-linker in mediating zippering. At the leading edge of epithelial cells, Shot regulates protrusion dynamics by promoting filopodia formation. Detailed cell biological analysis revealed that *shot* controls the morphology of the MT network in the epithelial cells by regulating dynamic properties of microtubule growth. FRAP (Fluorescence Recovery After Photobleaching) analysis and *in vivo* imaging of microtubule growth revealed that Shot stabilizes dynamic microtubules. In this process, Shots actin and microtubule binding activities are simultaneously required in the same molecule indicating, that Shot is engaged as a crosslinker.

We propose that in epithelial cells *shot* coordinates the interactions of distinct cytoskeletal components, which enables the cells to rapidly restructure their cytoskeleton and adopt an appropriate organization to the physiological requirements of the closure. Shot mediated interactions between microtubules and actin filaments facilitate formation of filopodia, which promote zippering by initiating contacting of opposing epithelial cells during zipping. Our work provides insights, how mechanisms integrating individual cytoskeletal elements into complex, highly shaped functional patterns contribute to a developmental process at the organism level.

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Growth regulation by the *Arabidopsis* E2FB transcription factor: cell cycle dependent and independent regulatory mechanisms

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Growth in plants is determined in specific regions called meristems, by coordinating the balance between cell proliferation and differentiation. The conserved retinoblastoma-related RBR tumour suppressor, together with its downstream effector E2F transcription factors operates in this balance by regulating either positively or negatively the underlying transcriptional programs. Model plant, *Arabidopsis thaliana* has a single RBR and three E2Fs (E2FA, E2FB and E2FC) that have the ability to interact with RBR. E2FA and E2FB are thought to function as transcriptional activators, while E2FC acts like repressor.

According to its expected role, we have found that E2FB is functioning as an activator of cell cycle genes in actively proliferating pavement cells in young developing leaves. We show that E2FB regulates RBR both transcriptionally and on protein level, but also controls its phosphorylation level. In accordance, CYCD3,1 transcript level was elevated in the ectopic E2FB lines. These feedbacks could provide the underlying mechanism, which controls the switch from cell proliferation to cell cycle exit and differentiation. In contrast to pavement cells, co-overexpression of a mutant E2FB$^{\Delta RBR}$ (unable to transactivate and form complex with RBR) with dimerization partner A (DPA) expands the transit amplifying cells produced by leaf meristemoid stem cells. In addition, reducing E2FB level by artificial microRNA technique causes extra divisions of the meristemoids resulted in stomata clusters. Thus E2FB has dual functions regulated by RBR. RBR represses E2FB activity, dependent on its phosphorylation, regulating cell cycle exit in pavement cells, E2FB forms a repressor complex with RBR to regulate stem cell divisions in meristemoids.

We found that E2FB regulates the non-cell cycle Leafy cotyledon 2 (LEC2) and Wrinkled1 (WRI1) transcription factors. Originally, identified as regulators of embryo and seed development. Expression analysis reveals that E2FB down regulates these genes might be in complex with RBR during seed development. Unexpectedly, LEC2 gene was found to be active in the root within the stem cell region of young seedlings and this pattern was significantly attenuated when its putative E2F-binding site was mutated. WRI1 regulates the accumulation of nutrient reserves of seeds, and its mutation resulted in oil-free small and wrinkled seeds, unable to leave the embryonic developmental stage unless they were grown in the presence of externally added nutrients (sucrose). Increasing the expression of WRI1 in its own expression domain resulted in improved growth, but the strongest expressing lines also show developmental defects on a sucrose-dependent manner. We have identified a putative E2F-binding site in the promoter-region of WRI1, and its mutation caused embryo and young seedling developmental abnormalities related to failed auxin signalling (*e.g.,* monocotyledonous embryo). Therefore, we suggest that these transcription factors have regulatory roles in plant development not identified yet and these functions are under the control of the E2F-RBR pathway.

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Biological effects of β-sheet analogs designed on the basis of synthetic oligopeptide anginex

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The new main line of pharmaceutical research is development of drugs, which based on peptides. Peptides have number of advantages compared to proteins. They are highly specific, water soluble and can be produced easier than complete proteins. Anginex is such an artificial peptide, which was designed by Kevin Mayo and co-workers. It was synthesised on the basis of the sequence and three dimensional structure of endogenous proteins such as platelet factor-4 and bactericidal permeability increasing protein. Similarly to the parent proteins it has β-sheet structure. Furthermore, it has biological activity, which is unique among the artificial peptides with β-sheet structure.

However, our experiments showed that anginex is highly aggregated in solution probably due to its β-sheet structure, furthermore according to the literature its half-life *in vivo* is likely short. Therefore, we started to design anginex analogs in which specific amino acids in β-sheet structure were substituted by β-amino acids or β-amino acids with cyclic side-chains. These new constructs did not possess the above mentioned disadvantages of anginex. Our experiments aimed to detect, whether anginex analogs preserved the functional properties of anginex, and characterize the mechanism of anginex effect.

Effects of the molecules were tested on cell viability of a murine brain microvascular endothelial cell line, bEnd.3. A strong reduction was observed in case of the cell viability after treatment with anginex and a few analogs. Determining the mechanism of the inhibition in the viability test further studies with anginex were extended investigating its effect on cell proliferation and cell death. These results showed that anginex was toxic to the cells, instead of inhibiting the cell proliferation or inducing apoptosis of bEnd.3. According to literature, biological activity of anginex is based on its interaction with the cell membrane. In order to gain a greater insight of the peptide–membrane interactions, membrane leakage assay was carried out by using carboxyfluorescein (CF) dequenching. Our results showed that anginex and those peptides, which were effective in the viability assay also increased the CF release from the micelles.

Our data suggest that effectiveness and secondary structure of anginex can be preserved by certain substitutions, and the inhibition of cell viability after peptide treatment is the consequence of their membrane damaging property.

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Identification of potentially bioactive peptaibol compounds from *Trichoderma* strains

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A large number of economically important antibiotics, secondary metabolites and extracellular enzymes – including peptaibols, a unique peptide group of antibiotics – are produced by species of the filamentous fungal genus *Trichoderma* belonging to the *Hypocreales* order of Ascomycota. Species of this genus are known as important sources of antibiotics and enzymes, and they are also known as promoters of plant growth, decomposers of xenobiotics and as commercial biofungicides. However, *Trichoderma* strains have also been reported in an increasing number of cases as etiologic agents in human infections and as causal agents of green mould epidemics in the commercial production of mushrooms.

Peptaibols are linear, amphipathic oligopeptides consisting of 5-20 amino acids with characteristic molecular weights of 500-2200 Dalton (Da). They are usually containing nonproteinogenic amino acid residues, like isovaline (Iva) and -aminoisobutyric acid (Aib) as characteristic building blocks of their structure, an acetylated N-terminus, and an amino alcohol, mostly phenylalaninol or in certain cases valinol, leucinol, isoleucinol or tryptophanol is linked by a peptide bond at the C-terminal end. The name peptaibol derives from the words peptide, Aib, and amino alcohol. The peptaibols produced by *Trichoderma* species regularly comprise 18-20 amino acid residues, and their unusual amino acid content is synthetized through non-ribosomal biosynthesis. The bioactivities of peptaibols are mainly related to formation of channels in lipid membranes, which are containing several hydrophobic transmembrane helices surrounding a central pore. The structure, microheterogenity and biological effects of these molecules were investigated over the past 45 years. The structure and properties of a considerable part of more than 1000 peptaibiotics are presented in the Comprehensive Peptaibiotics Database, which is freely available from the internet at http://peptaibiotics-database.boku.ac.at.

At the beginning of my work, alamethicin F50 – the firstly described and most studied peptaibol was investigated by HPLC-MS (High Performance Liquid Chromatography–Mass Spectrometry) instrument and an effective, easy method was developed for the analysis of the chromatograms and spectra. Subsequently, 59 different *Trichoderma* strains were cultivated on malt extract agar medium and the produced peptaibol compounds were extracted with chloroform, evaporated and finally dissolved in methanol. These crude extracts of the strains were investigated by using the method described above. Among the crude extracts of the 59 strains, 54 produced different kinds of peptaibols, which can be classified into the groups of trichorzianins, the MA and PA subgroups of trichorzins, trichobrachins, trichostrigocins, and a yet undescribed, new group of peptaibols. In several cases, previously described peptaibol sequences were identified, furthermore in certain cases new sequences were found and identified. In 90% of the cases the complete sequences were determined without identifying amino acid isotypes. The sequences of the peptaibols were compared with each other, and the stabile and labile parts of the sequences proved to be useful during the further studies.

The purification of the peptaibols was performed by semi-preparative HPLC (High Performance Liquid Chromatography*)*. The purified samples were examined by HPLC-MS, and the chromatograms and spectra were analyzed. After the separation of the compounds the isotype of the amino acids in the sequences can be identified, but for this a higher quantity of peptaibols is required. An entirely new cultivation method is under development in order to reach high production.

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Resistance mechanisms in *Candida parapsilosis*

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The number of invasive candidiasis has massively increased over the last decades and the currently available antifungal therapies often fail to control these disease. *Candida albicans* is the most frequently isolated species from candidemia, however, the infections by other “non-albicans” species, such as *C. parapsilosis* cause severe problem in many hospitals, recently.

Candida infections are typically treated with triazoles, the inhibitors of ergosterol biosynthesis, or echinocandins that inhibits beta-glucan synthesis in the fungal cell wall. The intensive use of such antimycotic drugs can lead easily to resistance development. While there has been a great progress in the understanding of the molecular mechanisms of drug resistance in *C. albicans*, we have little information about these mechanisms in *C. parapsilosis.*

The rapid expulsion of drugs by transporters belonging to ABC (ATP binding cassette) super-families, represent one of the prominent mechanisms of multidrug resistance in *Candida* clinical isolates. While the role of ABC transporters Cdr1 and Cdr2 in *C. albicans* is well documented, the role of these channels in *C. parapsilosis* remains unexplored. Thus, our aim was to study CpCDR1 and CpCDR2 and explore their role in cellular function with special focus on resistance and virulence. We generated *CDR1* and *CDR2* double knock out mutants and examined the fluconazole sensitivity and virulence properties. Interestingly, double deletion mutants showed not only increased sensitivity to fluconazole but also displayed an increased survival in both *in vitro* and *in vivo* infection models.

To further examine the correlation between resistance and virulence, we performed a microevolution experiment where we grow *C. parapsilosis* in the presence of sub-inhibitory concentration of fluconazole and echinocandins to provoke adaptation to antimycotics. Using this approach we were able to generate *C. parapsilosis* lines that were adapted to antimycotics at a significant level. Next, we will examine the virulence properties of the “evolved” strains and explore the possible link between virulence and adaptation to drugs.

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How the molecular environment affects the structure and function of plant light-harvesting complex II

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In photosynthesis absorbed light energy is collected by the so-called light-harvesting antenna complexes and delivered, by way of fast and efficient excitation energy transfer (EET), to the photochemical reaction centres, *e.g.,* in Photosystem I (PSI) and Photosystem II (PSII).

The antenna functions largely depend on the molecular architecture of the pigment-protein complexes and their organization in the thylakoid membranes, which is exploited by the photosynthetic machinery to dynamically tune light harvesting in response to the physiological conditions. Light-harvesting complex II (LHCII), the main antenna complex in plants, plays key roles in the regulation of light harvesting, *e.g.,* by dissipating the excess absorbed energy as heat or by migrating between PSII and PSI balancing the excitation energy flow (state transitions). To gain better understanding of these functions, we studied the structural and functional characteristics of LHCII *in vitro* paying particular attention to how they are altered by changing the molecular environment of the complex.

Extraction of the LHCII from the native membrane by the use of surfactants brings about significant changes in its structural organization and function. We used circular dichroism (CD) spectroscopy to distinguish the effects of protein-protein contacts, lipids and detergents on the pigment-pigment exciton interactions in LHCII. Comparing the CD spectra of LHCII in detergents, aggregated, in lipid membranes or trapped in polymer gel, we identified spectral changes specific to LHCII-LHCII interactions and to the interaction with detergent or membrane lipids. Our results confirmed that the structure of the complex is sensitive to the molecular surrounding; for example, there was indication that the detergent micelle alters the conformation of the carotenoid neoxanthin. Furthermore, we employed anisotropic CD of macroscopically-aligned LHCII, a novel approach in photosynthesis, to discriminate between excitonic transitions having different orientations with respect to the membrane plane.

The effect of the environment on the functionality of the complex, i.e. the excited-state lifetime and energy transfer dynamics was tested by ultrafast time-resolved spectroscopy techniques – time-resolved fluorescence, femtosecond transient absorption and two-dimensional electronic spectroscopy (2DES). Confirming previous studies by other groups, our results showed that the lifetime of excited chlorophyll in LHCII strongly depends on the environment (from 4 ns in detergent micelles to 0.2 ns in aggregates) and that quenching in LHCII aggregates is activated by the aqueous environment rather than aggregation itself. We further revealed, by 2DES, that small structural changes in LHCII in the aggregated state lead to alteration of the dynamics and pathways of energy transfer between chlorophylls.

In contrast to aggregates, reconstituted LHCII-lipid membranes showed that excitations lived long enough (2 ns) to enable efficient light harvesting function. Moreover, excitation energy could migrate over several LHCII complexes in the membranes, providing degree of energetic connectivity similar to native thylakoid membranes. We took advantage of this characteristic to test another known function of thylakoid membranes, namely the ability of LHCII to donate excitations to PSI. To this end, we reconstituted membranes with LHCII and PSI at different stoichiometric ratios and monitored energy transfer by steady-state and time-resolved spectroscopy. Different pools of LHCII were found to transfer energy to PSI on time scales from less than 10 ps to hundreds of ps, contributing significantly to the effective antenna size of PSI. The overall efficiency of transferring excitations from LHCII was up to 70%. Moreover, due to the remarkably efficient charge separation in PSI, the overall photochemical quantum yield remained very high, demonstrated the ability to construct artificial systems with a desired functional antenna size without a significant loss in quantum efficiency.

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"On the traces of tuberculosis" – Possibilities of macromorphological diagnosis of tuberculosis in prehistoric osteological series

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Nowadays, tuberculosis (TB) is one of the most lethal infectious diseases that attacks with renewed vigour after a few years of regression in the 1970's. According to the estimate of the WHO, there are approximately 2 billion people in the world infected with the causative *Mycobacterium* species, which is nearly one third of the world's current total population. The most common form of the disease is pulmonary TB, but in the minority of the cases (about 15–20%) the bacteria spread from the initial site of infection to other compartments of the human body, including the skeletal system, resulting in extra-pulmonary TB. Data indicate that about 3–5% of patients with chronic TB suffer from osseous involvement.

My PhD thesis project is targeted to the differential diagnosis of skeletal TB in paleopathological settings with special emphasis on macromorphological diagnostic methods. Traditionally, the macromorphological diagnosis of skeletal TB in human remains is based upon the detection of secondary skeletal lesions associated with tuberculous infection. At the beginning of TB research, macromorphological examinations were focused only on the registration of the so-called classical/advanced-stage TB alterations (*spondylitis tuberculosa* and tuberculous arthritis), representing a fairly developed stage of TB. However, the prevalence of skeletal TB must have been remarkably higher in the past than it was suggested on the basis of these previous investigations, because TB may have affected many individuals without classical TB changes, thus patients died in an earlier stage of TB long before these symptoms could have developed. Clearly, this early-stage TB is not

recognisable on the basis of classical lesions. Because of the problems of TB diagnostics, the importance of establishing diagnostic criteria for early-stage TB became recognised in the late 20th century. A number of studies have focused on searching for atypical or early-stage lesions in connection with TB infection. As a result of these investigations, three types of atypical/early-stage TB alterations gained more attention: rib lesions, superficial vertebral changes, including hypervascularisation, and endocranial alterations. Although there seems to be a positive correlation between these lesions and TB, they are not always specific for the disease. However, the simultaneous occurrence of several of these alterations can increase the probability of the diagnosis of skeletal TB.The aim of our study is to summarize the results of the TB paleopathology research in the Szeged Anthropological Collection from the 1970's to the present day, focusing on the distribution of different types of TB-related skeletal lesions. We also intend to draw attention to the importance of re-examination of previously analysed osteological samples with uniform application of classical and atypical traits in the diagnosis of skeletal TB.

The skeletal material for this study derives from 44 archaeological sites from the Neolithic period to the Late Middle Ages, totalling 3906 individuals. The macromorphological investigation was based upon the detection of both classical and atypical TB changes. During the macromorphological analysis of the skeletal material, 497 cases of probable skeletal TB were detected. Classical TB lesions were observed in 28 cases, while atypical TB alterations were registered in a further 469 cases.

According to our results, TB could be a major infectious disease in past populations in Hungary. New cases deriving from prehistoric osteological series are of particular importance, because they are among the oldest paleopathological cases of TB in Hungary. Moreover, our data contribute to strengthen the importance of uniform application of classical and atypical traits and also provide a promising basis to carry on with this research direction.

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Occurrence of toxin-producing fungi and the level of resistance against these pathogens in maize

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Maize (*Zea mays* L.) is the most important ingredient of feeds, and one of the most important agricultural export product of Hungary. Food and feed safety problems have increasing significance in maize production. Many fungal pathogens are able to infect maize, and many of them are able to cause mycotoxin contamination. The most important mycotoxin producers are members of the *Fusarium* and *Aspergillus* genus. Although *Fusarium* toxins, including trichothecenes, zearalenone and fumonisins, are considered to be the most important in cereals in regions with temperate climate. Mycotoxins produced by Aspergilli are also frequently identified in cereal products (Halt et al. 2004; Giorni et al. 2007; Tabuc et al. 2009) and recently also in Hungary. Among Fusaria the main toxin producers are *F. verticillioides* and *F. graminearum*, but many toxin producing species of smaller significance can also be found (Varga et al. 2004).

For *Fusarium* infections the moderately warm, humid weather is optimal at silking. The average summer temperature with higher humidity favors the spread of *F. graminearum*, but at a higher temperature *F. verticillioides* expansion can take place. Contrary, *Aspergillus flavus* requires especially dry and hot conditions to develop significant toxin contamination. Therefore, toxin contamination is a potential risk nearly every year. Maize products are frequently contaminated by fumonisins, which are principally produced by *Fusarium verticillioides*, although black Aspergilli, which are also able to produce fumonisin may occur on maize kernels. In this study, we examined the occurrence of these species and their mycotoxins in various maize growing areas of Hungary in two consecutive years. In 2013 the average fungal contamination was 50.1%. The rate of *Fusarium* species was 46%, 69% of them belonged to *F. verticillioides*, 28% *F. proliferatum*, 3% *F. subglutinans* and no isolate of *F. graminearum* was found. 9.6% of the isolated fungi belonged to genus *Aspergillus*. Most of these isolates belonged to the potential aflatoxin producer *A. flavus* and a smaller proportion was *A. niger* and *A. ochraceus*.

An infection rate of 71.4% of maize samples was observed due to the extremely high precipitation in 2014. The rate of *Fusarium* isolates was 84.9%. 44% of them belonged to *F. verticillioides*, 29% *F. graminearum*, 23% *F. proliferatum*, 3% *F. subglutinans* and 1% belonged to the species *F. sporotrichioides*. We couldn't isolate any *A. flavus* from the maize samples. *Aspergillus* contamination was not detected in the maize samples tested.

In 2012-2013 10 Hungarian and 10 Serbian hybrids with differing resistance were screened in Kiszombor (Hu) and Kapusina (Srb). The main task of this project was to screen the resistance level of maize hybrids against *Fusarium* and *Aspergillus* species. The hybrids were examined for resistance against *F. culmorum, F. graminearum*, *F. verticillioides* and *A. flavus*.

The hybrid differences were significant. The hybrids vary from very good resistance to high susceptibility to all of the fungal species following artificial inoculation. *F. graminearum* and *F. culmorum* were much more infectious than the other two toxic species.

F. verticillioides resistance correlates well with the *F. graminearum / F. culmorum* group, which was already mentioned in previous studies. (Mesterházy 1982; Mesterházy et al. 2000). However, in other experiments, there were several hybrids, in which resistance to *F. verticillioides* was only loosely bound, which means, that resistance must be examined in both groups. The genetic background of resistance to *Aspergillus flavus* is largely independent.

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Estrogen deficiency, physical exercise and diet-induced modifications in animal models of menopause: metabolic and inflammatory evaluations

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Pre-menopausal women have a lower incidence of cardiac and metabolic disorders compared to age-matched men, but this female advantage disappears after the onset of menopause. Furthermore, the manifestation of the diseases increases in post-menopausal women who have not used any therapeutic strategy. Abnormalities of metabolic components in menopause predict cardiovascular morbidity and mortality, which are the leading causes of death, therefore the development of different treatment strategies have become a public health priority.

The aim of our work was to investigate the effects of estrogen deficiency on inflammatory and cardiovascular parameters in various menopausal rat models and to determine the influence of lifestyle modifications in the treatment of menopausal symptoms.

The effects of estrogen deprivation were examined in pharmacologically ovariectomized (POVX, 750 µg/kg triporelin im. every 4th week, 4 month old rats), surgically ovariectomized (OVX, 4 month old rats), and 24-month-old ovary-intact (aged) Wistar rats. We measured the activity and expression of heme oxygenase (HO) enzyme in cardiac left ventricle (LV), the concentrations of cardiac interleukin-6 (IL-6) and tumor necrosis factor-α (TNF- α) pro-inflammatory cytokines, the myeloperoxidase (MPO) activity in the cardiac LV, and the effects of HO activity blockade (by 24-hour and 1-hour pretreatment with tin-protoporphyrin IX, SnPP) on the epinephrine and phentolamine-induced electrocardiogram ST segment changes (assessed by lead II. surface ECG) *in vivo*. To investigate the influence of lifestyle modification in experimental menopause, female Wistar rats underwent ovariectomy surgery (OVX) or sham operation (SO) and were fed standard chow (CTRL), a high triglyceride diet (HT) or a calorie restricted diet (CR). In addition to diet, rats were randomized into new groups based on the voluntary physical activity (with or without running) for 12 weeks. The metabolic syndrome was assessed by measuring the body weight gain, the glucose sensitivity, and the levels of insulin, triglyceride, leptin, and aspartate aminotransferase transaminase (AST) and alanine aminotransferase (ALT).

We found that estrogen depletion significantly decreased the cardiac HO enzyme activity and the expression of HO-1 and HO-2 isoforms; increased the expression of IL-6, TNF- α the MPO enzyme activity; and caused a significant ST segment depression. After pretreatment with SnPP augmented ST segment changes were determined. Exercise training combined with the CR resulted in improvements in the glucose tolerance and the insulin sensitivity. Plasma triglyceride, AST, and ALT levels were significantly higher in OVX rats fed with HT, but these high values were suppressed by exercise and CR. Similar results were obtained in relation to leptin values in the OVX HT group.

Our results demonstrate that the absence of estrogen led to an increase in inflammatory processes and metabolic pathways as well as resulted in the loss of cardioprotective response via HO enzyme regulation. CR combined with voluntary physical exercise can be a potential, non-pharmacological treatment in experimental menopause.

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Effect of the light and dark conditions on the salicylic acid-induced oxidative stress

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Phytohormones, such as salicylic acid (SA) are small molecules that are essential for the regulation of plant growth, development, reproduction and stress acclimation. SA is endogenous signalling molecule mediating local, hypersensitive response (HR) and systemic acquired resistance (SAR) against pathogens and tolerance to biotic stresses. SA accumulation, SA-mediated pathways, and host susceptibility to pathogens is dependent on light.

Polyamines [PAs (Putrescine, Put; Spermidine, Spd; Spermine, Spm)] are low molecular weight polycations, which also function in plant acclimation. Exogenously applied SA induced PA accumulation and it was able to activate the expression of biosynthetic genes, arginine decarboxylase (ADC), ornithine decarboxylase (ODC), spermidine synthase (SPDS) and spermine synthase (SPMS). The gaseous plant hormone, ethylene is synthesized from S-adenosylmethionine (SAM), which is also a precursor for the synthesis of higher PAs, Spd and Spm. Ethylene and PAs compete for the common precursor, thus they can inhibit each other synthesis. SA and ethylene signalling or PA catabolism by diamine (DAOs) and polyamine oxidases (PAOs)] may induce H_2O_2 production. H_2O_2 in high concentrations is essential mediator of HR and in excess amount it induces cell death. On the other hand, it can contribute to the maintenance of cellular redox homeostasis through the activation of antioxidant enzymes and can participate in regulation of other reactive oxygen species (ROS) and nitric oxide (NO).

It was found recently, that the development of plant immunity, the activation of several defence genes and regulation of the cell death response required light. Our work was focused on the effect of sublethal and lethal SA concentrations (0,1 mM or 1 mM, respectively) on PA metabolism in order to reveal the putative contribution of PA metabolism to oxidative stress in tomato leaf tissues under light and dark conditions. Since enzymes scavenging H_2O_2 is controlled by SA, we were curious to know how SA exerts a control on antioxidant enzymes in the light and dark environment.

The accumulation of exogenous SA was not different under light and dark conditions 24 h after SA treatment. The expression of genes involved in PA synthesis (ADC and ODC, SPDS and SPMS) exhibited SA concentration and light dependence. While PA biosynthetic genes were highly up-regulated by 1 mM SA, those participating in PA catabolisms, DAOs and PAOs displayed lower expression at 1 mM, but enhanced transcript level at 0,1 mM SA. As a result, Put and Spm content, but not that of Spd increased on 1 mM SA application, which proved to be higher in the dark than in the light. As it was expected, ethylene production was inhibited by high SA concentration under light conditions. From 6 h after treatment, 1 mM SA induced significant NO production in the light, but NO level declined under the dark. H_2O_2 content increased at much higher extent at 1 mM SA in the light, however, we did not find temporal coincidence with H_2O_2 accumulation at tissue level and terminal PA catabolism, which suggest that PA catabolism has only partial role in the generation of reactive oxygen species in photosynthesizing tissues.

Both SA treatments induced the activity of superoxide dismutase (SOD) and in parallel the expression of Mn- and Cu/Zn-SOD, but not that of Fe-SOD in the light. The activity and the expression of catalase (CAT) decreased after SA treatments independently of the illumination. Similarly, peroxidase (POD) activity was also light independent and increased in the presence.

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Isolation and characterization of a novel cysteine-rich, anti-yeast protein from *Neosartorya fischeri*

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The increasing incidence of infections caused by yeasts, which results serious problems in medicine. In the most cases *Candida* species are responsible for superficial and disseminated diseases in humans. Due to systemic *Candida* infections approx. 150 000 deaths are recorded in each year. Furthermore, the number of antibiotic resistant *Candida* strains is continuously increasing, which makes hard to treat these infections. Therefore, there is a substantial demand to develop new, anti-yeast compounds. Based on *in vitro* and *in vivo* investigations, cysteine-rich antifungal proteins secreted by filamentous Ascomycetes could be suitable for this reason. Until now, only three proteins have

been identified with a relatively weak anti-yeast activity from *Aspergillus niger* antifungal protein (ANAFP), *Penicillium brevicompactum* antifungal protein (BP) and *Fusarium polyphialidicum* antifungal protein (FPAP).

In this study, an anti-yeast protein was isolate and characterized from the ferment broth of *Neosartorya fischeri* NRRL 181. The *N. fischeri* antifungal protein 2 (NFAP2) NFAP2 was purified with cation-exchange chromatography and identified with mass spectrometric, molar mass measurement and mass spectrometric analysis of enzyme digested sample from the pooled fractions, which showed anti-yeast activity. Further 35 protein sequences with significant similarity to NAFP2 were identified in the genomic databases. Phylogenetic analyses clearly demonstrated that these proteins represent a new group of cysteine-rich antifungal proteins from Ascomycetes. *In silico* analyses revealed that the 5,6 kDa NFAP2 is constituted by 52 amino acids and its structure is stabilized by three disulfide bridges. Experimental investigations on the structure of NFAP2 are in progress. In susceptibility tests NFAP2 showed high antifungal activity with a MIC range from 0.13 to 1.56 µg/ml against yeasts involving clinically relevant *Candida* species.

After further, *in vitro* and *in vivo* investigations NFAP could serve a base to develop a new protein-based antifungal strategy against yeasts.

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The role of headcase in the hematopoiesis of *Drosophila melanogaster*

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Against microbial and eukaryotic parasites, the fruitfly (*Drosophila melanogaster*) possesses an effective immune response, which consists of humoral and cellular elements. The immune cells of *Drosophila*, the hemocytes, are located in distinct compartments and differentiate in multiple waves under the control of phylogenetically conserved epigenetic and transcription factors. Although the three larval hematopoietic compartments - the sessile tissue, the circulation and the lymph gland - have different mesodermal origin, they all contribute to the formation of the effector cells: the phagocytic plasmatocytes, the melanotic crystal cells and the capsule forming lamellocytes. In our screens, which were aimed to identify genes that play an important role in the control of the differentiation of hemocytes, we isolated *headcase* (*hdc*), the ortholog of the human tumor suppressor HECA, which is a repressor of differentiation in numerous developmental processes of *Drosophila*. We found that *hdc* is expressed in the lymph gland of naive larvae, however, upon immune induction the hemocytes leaving the organ differentiate into lamellocytes and lose *hdc* activity. This suggests that *hdc* may be a regulator of hemocyte differentiation.

We carried out P-element conversion and P-element remobilization screens to generate *hdc-Gal4* driver lines and amorphic *hdc* allele. Our newly generated *hdc-Gal4* driver and numerous hemocyte specific transgenic drivers were applied to silence *hdc* by RNA interference and followed the hemocyte differentiation by the use of immunological markers and *in vivo* transgenic reporters. We performed a candidate misexpression and silencing screen to identify the interaction partners of Hdc. The prepared samples and the hematopoietic compartments were analyzed with fluorescent and *in vivo* confocal microscopy.

In the lymph gland, *hdc* expression was not restricted to hemocytes, but was also observed in the Posterior Signaling Center (PSC), the hematopoietic niche that blocks the differentiation of hematopoietic progenitors in this compartment. The hypomorphic and amorphic mutations of *hdc* caused spontaneous lamellocyte differentiation. By silencing *hdc* with various hemocyte specific drivers, we mapped the focus of the mutation to the PSC of the lymph gland. In a PSC-specific misexpression screen, we identified the members of the hedgehog pathway as potential interacting partners of Hdc.

Our results show that Hdc - expressed in the PSC cells - plays a regulatory role in the differentiation of lamellocytes through its interaction with the Hh pathway. The nonautonomous effect of the factor suggests a potential crosstalk of the hematopoietic compartments with unknown molecular background.

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Experimental test of the genome streamlining hypotheses

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Evolution of genomes is characterized by long, dominant periods of genome reduction, punctuated by short, rapid expansions. Understanding the driving forces of the processes shaping genome complexity is of paramount importance in the era of designing and constructing semisynthetic microbial genomes. Hypotheses on genome evolution, and on the role of adaptation and drift in it, are mostly conjectures based on bioinformatic analysis of available genome sequences. Experimental testing of the hypotheses is hindered by the slow pace of natural genome evolution, and by the lack of tools speeding up large-scale genome evolution in the laboratory. We propose a novel scheme of random deletion generation to accelerate laboratory evolution of the *E. coli* bacterium, and address the validity of the genome evolution hypotheses. Taking advantage of the fact that the double-strand DNA break repair in *E. coli,* is associated with the formation of deletions. We plan to generate a large number of random genomic deletions in a cyclic fashion in parallel cell lines, under various environmental conditions. Analysis of the fitness and the genome sequence of the strains, selected by their growth advantage, will reveal the driving forces of natural genome reduction and shed light on the genome organization principles. Furthermore, it will produce a blueprint for a minimal *E. coli* genome.

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Essential oils as alternative disinfectants in the food industry

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Bacteria can attach to different surfaces and form biofilm. In the bacterial matrix cells are more resistant to sanitizers than in free swimming form. If the used chemicals cannot guarantee elimination, biofilms could contaminate food and cause food borne diseases. Moreover, changes in consumer's behavior turned producers to green technologies in food processing so it became necessary to search for new, natural antibacterial agents. Essential oils (EOs) are plant derived hydrophobic substances and have antibacterial, antiviral and antifungal effects.

In our study the antibacterial, anti-adhesion and disinfection effect of cinnamon, clary sage, juniper, lemon, marjoram and thyme EOs on food spoilage bacteria (*Escherichia coli, Pseudomonas putid*a), and pathogens (*Bacillus cereus, Bacillus subtilis, Listeria monocytogenes*, methicillin-sensitive and resistant *Staphylococcus aureus*) from the Szeged Microbial Collection and on food industrial isolates was investigated.

Our experiments had four parts: (1) determination of minimum inhibitory concentration (MIC), minimum bactericide concentration (MBC) and killing time on microbes from the microbial collection; (2) determination of (MIC), (MBC) and killing time on isolates; (3) investigation of anti-adhesion effect of EOs on native and with food residues coated industrial surfaces (polypropylene, stainless steel); (4) evaluation of the disinfection effect of EOs on 1 and 7 days old biofilms.

Most of the investigated EOs had good antibacterial effect, with the lowest MIC and MBC for cinnamon, marjoram and thyme. Lemon was excluded from further research, because it had no effect even at 100 mg/ml concentration. Killing time was optimized using Box-Behnken Response Surfaces Design with the independent variables of concentration, time and pH. At acidic pH the achieved disinfection time (< 30 min) is suitable for the food industry. The investigated essential oils eliminated mono- or co-cultured (*E. coli, L. monocytogenes, P. putida, S. aureus*) bacterial biofilms from industrial surfaces in 30 min, when the disinfection solution pH was 4.5 or lower.

The investigated EOs were able to reduce bacterial adhesion, but remained food residues diminished this effect. It is hypothesized that volatile components of EOs bind to animal protein and lost their activity. Marjoram and thyme EOs prevented totally the attachment of methicillin-resistant *S. aureus* to both surfaces.

From pastry and meat industries microbes belonging to the genera *Escherichia, Pseudomonas, Serratia, Enterobacter, Citrobacter* were isolated. Cinnamon, marjoram and thyme EOs proved to be good antimicrobials: MIC and MBC were higher and killing time was shorter compared to the cultured microbes.

Based on our results EOs had good disinfection effect. Essential oils are natural, eco-friendly and volatile, thus rinsing is not necessary at the end of hygienic process. EOs can represent an alternative way for disinfection.

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Ascorbate biosynthesis and its regulation by *VTC2* in the green alga *Chlamydomonas reinhardtii*

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Ascorbate (Asc) is of vital importance to the cellular functions of both animals and plants. It is an essential scavenger of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$) and superoxide and it is also a cofactor of several 2-oxoacid-dependent dioxygenase enzymes, which catalyse a large number of physiological processes in the cell. In higher plants, besides its role as an antioxidant, Asc also participates in cellular development and synthesis of the cell wall. Asc also modulates the synthesis of several signalling molecules such as abscisic acid, gibberellins, ethylene and salicylic acid. It also influences anthocyanin accumulation during high light acclimation. It is involved in the regulation of stomatal movement and also modulates the expression of specific sets of photosynthesis and defence genes via poorly understood mechanisms. Asc also contributes to the non-photochemical quenching in higher plants, because it is also a cofactor of violaxanthin de-epoxidase. Besides, it is an alternative electron donor to photosystem II and we showed recently that in the green alga *Chlamydomonas reinhardtii* Asc may regulate photobiological H_2 production under sulphur deprivation. In plants, the main route for Asc biosynthesis is the L-galactose (Smirnoff-Wheeler) pathway. In *Chlamydomonas reinhardtii* direct physiological and molecular evidence for the operation of the Smirnoff-Wheeler pathway is still lacking, in the sense that no mutants affected in Asc biosynthesis have been generated or investigated so far. In order to elucidate Asc biosynthesis in green algae, we decided to generate Asc-deficient *Chlamydomonas* transformants. As a target, we have chosen the *VTC2* gene, encoding GDP-L-galactose phosphorylase, since in higher plants it is known to be highly regulated and its activity has a strong influence on the Asc content; gene expression studies on *Chlamydomonas* suggested the same importance. For the gene silencing, we used the artificial microRNA (amiRNA) approach since it is highly specific in *Chlamydomonas* and overcomes the problem of self-silencing as observed for the siRNA approach. The amiRNA to target *VTC2* was introduced into the CW-15-325 wild type strain that has been successfully used before for generating amiRNA lines. Our *VTC2-amiRNA* lines have about 10% Asc relative to the control strains showing that GDP-L-galactose phosphorylase plays a pivotal role in Asc biosynthesis. The *VTC2-amiRNA* lines also grow more slowly, have lower chlorophyll content and smaller light-harvesting antenna. Under normal conditions, the *VTC2* expression level is down-regulated in the *VTC2-amiRNA* lines, just as well as the GDP-L-galactose phosphorylase content. However, upon H_2O_2 treatment, the *VTC2* expression is strongly increased in the *VTC2-amiRNA* lines, which is not accompanied by a proportional increase in the GDP-L-galactose phosphorylase content. As a result, the *VTC2-amiRNA* lines are more susceptible to H_2O_2 and other stress treatments than the control strains. We have also shown that, as opposed to higher plants, in the absence of photosynthesis, oxidative stress (via H_2O_2 or $^1O_2$) by itself can induce a significant increase in Asc content, indicating that Asc biosynthesis in *Chlamydomonas* is probably regulated via oxidative stress signalling and it is independent of photosynthesis.

Finally, we have demonstrated that Asc biosynthesis is not regulated by a circadian clock and there is a positive feedback of Asc on the *VTC2* expression enhancing its own biosynthesis.

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In vivo recognition of various cyanobacterial PS I oligomeric forms monitored by cd spectroscopy

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Photosystem I (PS I), is the largest pigment-protein complex in photosynthetic membranes. It catalyzes the electron transfer from reduced plastocyanin on the lumenal side of the membrane to ferredoxin or flavodoxin on cytoplasmic/stromal membrane side. PS I of algae and higher plants exists exclusively in monomeric form. While cyanobacterial cells contain PS I organized as monomers and trimers. In photosynthetic membranes of *Synechocystis* PCC6803, a dynamic equilibrium exists between the monomeric and trimeric forms of PS I. Trimeric form may increase the cross section of light absorption, which can help the light harvesting process at the longer wavelength region. The equilibrium between PS I monomers and trimers is very sensitive to *in vitro* experimental methods. In order to monitor PS I oligomerization state, we used a noninvasive, *in vivo* method called circular dichroism (CD) spectroscopy, which is suitable to detect molecular changes in isolated PS I complexes, thylakoid membranes and even living cells. We found, the CD spectrum is dominated by excitonic signals originating from PS I in cyanobacteria. Overlapping excitonic band of carotenoid and chlorophyll cromophores results in a complex CD spectra, therefore, we need to identify the CD fingerprints of various PS I oligomerization forms under different physiological conditions

of various cyanobacterial strains. We performed a comparative CD spectroscopic study on a trimer deficient psaL mutant and wild type *Synechocystis* PCC6803 cells and thylakoid membranes. We observed a marked discrepancy between the spectrum of the two strains in the Soret region at the peak maximum of 510 nm. However, this band was absent in the carotenoid-less mutant (crtB) confirming that this peak is due to an unidentified specific carotenoid. In the wild type the majority of PS I are trimeric forms and their peak maximum, at around 510 nm is significantly higher than in the psaL mutant.

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