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DISSERTATION SUMMARIES

Circularly permuted variants of two CG-specific prokaryotic DNA-methyltransferases

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The prokaryotic cytosine (C-5) DNA methyltransferases (MTase) M.SssI and M.MpeI share the sequence specificity of mammalian MTases, thus they are excellent research tools for studying certain aspects of mammalian CpG-methylation. Both M.SssI and M.MpeI are amenable to fragment complementation, *i.e.* certain inactive truncated fragments of the proteins can assemble to form active enzyme. Our work with fragment complementation was hindered by the instability and/or low solubility of C-terminal fragments of M.SssI and M.MpeI. We assumed that poor solubility was due to the exposure of the hydrophobic C-terminal α-helix to the solvent. We hypothesized that this problem could be circumvented by using fragments derived from circularly permuted (CP) enzyme variants. Genes encoding CP variants of M.SssI and M.MpeI were created by PCR using tandemly duplicated MTase gene copies as templates. Most of the permutation sites were designed to leave conserved motifs and known secondary structural elements intact. MTase activity of the permutants was tested by a restriction protection assay and by measuring the incorporation of tritiated methyl groups into DNA. Eleven of the fourteen cpM.MpeI and six of the seven cpM.SssI variants were shown to have detectable MTase activity. Based on the linear arrengement of the conserved motifs, the catalitically active permutants were classified in ten topological types (A - J). Type B permutants, in which the new N-termini are located between conserved motifs II and III, had by far the highest activity. A computer search of the C5-MTase sequences available in the REBASE database revealed several MTases with naturally CP sequence. Interestingly, they appear to represent only one of the CP topologies created in this work. To our knowledge this is the first study describing the construction of designed CP C5-MTases. The wide range of CP topologies compatible with detectable MTase activity is a new evidence for the structural plasticity of C5-MTases. The CP variants open new possibilities for enzyme engineering and for testing the available structural models of the two enzymes.

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Functional characterization and subcellular localization of CDPK Related Kinase (CRK) family in *Arabidopsis thaliana* plant

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The CDPK (Ca^{2+}–dependent serine/threonine protein kinases) superfamily existing only in plants consists of several subfamilies like CDPK family and the structurally closely related CRK (CDPK-Related Kinase) family. Within the CDPK superfamily, the CDPK subfamily is widely involved in regulation of several abiotic and biotic stress responses in diverse plant species which is up to now relatively well documented. However, functional role of the CRK subfamily (CRKs) which contains eight members in *Arabidopsis thaliana (At)* are the least characterized until now. Previous studies from one of the members of AtCRKs showed that the plasma membrane localized AtCRK5 is required for proper polar localization of PIN2 in *Arabidopsis* roots. Inactivation of AtCRK5 causes root gravitropic defect; reduced root growth and enhanced lateral root formation. In this study, we performed the functional analysis of T-DNA insertion mutants of *Arabidopsis* CRK family members and over-expressing transgenic lines tagged with Green Fluorescent protein (GFP) and further their characterization of developmental alterations, mostly their response to gravitropic processes in roots/hypocotyls bending and root growth. Studies of the AtCRK family members with C-terminal GFP tag revealed that most of the members exhibit plasma membrane localization in the roots as was predicted by their N-terminal myristolyation sites and thus assumed to be important candidates for study of root gravitropic and other growth responses. Delayed root gravitropic and hypocotyl bending in most of the At*crk* T-DNA insertional mutants was observed when compared to the wild type (Col-0). Furthermore, the in-depth characterization of AtCRK1, which was earlier reported to be thermo-tolerant and salt sensitive member of this family, was found to be photo sensible

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in continuous light growth conditions which lead to its dwarf phenotype and enhanced cell death in leaf tissues revealing its potential regulatory role in maintenance of cellular homeostasis during continuous light conditions.

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The biological significance of the nuclear localization of an actin-binding cytoskeletal protein

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The members of Ezrin-Radixin-Moesin family of proteins play important role in cytoskeletal rearrangements. They act as crosslinkers between membrane proteins and the actin cytoskeleton, thereby function in cell migration and metastasis. The majority of ERM proteins localize in the cytoplasm and to the cell cortex, however the Moesin (Moe) protein of *Drosophila melanogaster*, the only representative of the ERM protein family in the fly, has been detected by our laboratory also in the cell nucleus. The cytosolic role of Moe is well characterized but since its nuclear transport mechanism is unknown, the direct study of its nuclear function is not possible. To overcome this problem and to eliminate the nuclear functions of the Moe protein without affecting its manifold cytoplasmic functions, we aimed to tag the *Moe* gene *in situ* with a nuclear export signal (NES) by applying the genome editing CRISPR-Cas9 method. As a result, the Moe-NES protein would be constantly cleared out from the nucleus while it can still perform its cytoplasmic functions.

To achieve our goal, we designed and tested the efficiency of the guide-RNA (gRNA) constructs in Drosophila embryos and built up the donor construct which served as a template for homologous recombination. Next, we co-injected Cas9 producing embryos with the gRNAs and the donor construct, then screened for succesful recombination. Four *Moe*[NES] mutant lines have been recovered. Mutant adult flies showed dominant grandchild-less phenotype: germline stem cells and germ cells are both missing from the ovaries. We also observed developmental defects in *Moe*[NES] animals: bristle and wing phenotypes, cuticular and bristle malformations and the rotation of genitalia. The grandchild-less phenotype turned out to be of maternal origin while the developmental defects are zygotic effect phenotypes. Besides developmental defects, *Moe*[NES] flies also exhibit decreased heat shock tolerance and climbing capacity. We plan to reveal the molecular mechanisms behind these phenotypes by the detailed examination of the ovary and embryos of the grandchild-less females.

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A bioarchaeological analysis of horse riding in the Hungarian conquerors

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In certain conditions, some changes observed on the bones can be related to an intense and regular physical activity during the life of individuals. The reconstruction of the activities in past human societies is considered by some scholars as the "Bioarchaeology's Holy Grail". However, due to various biases and the lack of a clear cultural context, the link between skeletal markers and specific activities cannot often be reliably determined. Horse riding, in particular, has already been investigated in bioarchaeology, but what markers should be considered as specific for this activity is still a debated discussion. This study is an attempt to clarify the question, from the analysis of skeletons of archaeologically presumed horse riders.

The Hungarian tribes who conquered the Carpathian Basin from the end of the IXth Century and during the Xth Century CE were composed of powerful armies of archers mounted on horses. In many cemeteries from the Conquest period, we can frequently find archery and horse riding equipment or horse bones, intentionally deposited in the graves.

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We relied on the rich cemetery of Sárrétudvari-Hízóföld (Hungary), with a total of 263 burials, and performed a systematic macromorphological analysis on the adult skeletons, including scoring of joint, muscular insertion, and vertebral changes, as well as morphological variations, traumas, and a large series of infracranial measurements. We present here part of the results, with a special focus on the coxofemoral joint, and we attempt to correlate the observed skeletal changes with the presence of horse riding equipment in the graves.

This study aims to provide a methodological contribution in the field of activities in past populations, for reliable identification of the presence of riders in anthropological collections, as well as to improve our understanding of the societies of Hungarian conquerors in particular.

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Production of bioactive phenolic compounds from fruit residues by solid state fermentation and carbohydrase treatment

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Application of plant derived phenolics in functional foods has increased on the past years. Though many of these phenolics have antioxidant and antimicrobial capacities, their bioavailability is often limited due to the glycosidic complexes formed. Carbohydrate-cleaving enzymes, however, can hydrolyze these bonds releasing the phenolic aglycone. Fruit residues are excellent substrates for the production, thus, we aimed to mobilize such bioactive phenolic compounds from oven-dried and lyophilized white and black grape, apple, pitahaya (known as dragon fruit), mango, naranjilla and tomato residues via two approaches: i) *in vivo* solid-state fermentation with the cellulolytic fungus *Rhizomucor miehei* NRRL 5282 and ii) *in vitro* enzymatic treatment using *R. miehei* cellulase and *Aspergillus niger* pectinase cocktails.

Positive correlation between the total phenolic content and antioxidant activity was generally found after the fermentation and the enzymatic treatments. However, the antioxidant activity increase depended on the substrate pretreatment technique as well. Concentration of the major individual phenolics determined by HPLC changed by different degrees after the enzymatic treatments depending on the substrate and the pretreatment. In further studies, the antimicrobial and antibiofilm activities of the enzyme treated extracts were evaluated against foodborne pathogens and food spoilage bacteria. Then, we studied the anti-quorum sensing potential of the samples using the model organism *Chromobacterium violaceum*. In general, carbohydrase treatments influenced positively the anti-QS properties of the extracts as well. The effects were strongly depended on the type of the fruit, the pretreatment and the enzymatic treatments. Black and white grapes displayed the highest antimicrobial activity among the residues tested, while the mango samples showed the most significant effect against the bacterial biofilm formation. The tomato and naranjilla samples were outstanding in terms of the anti-quorum sensing activity. For all extracts, the sensitive bacteria were among the *Bacillus*, *Pseudomonas* and *Staphylococcus* strains and the resistant were the *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli*.

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Do maternal smoking during pregnancy alters the morphology, rheology and function of red blood cells?

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Cigarette smoking during pregnancy imposes risks to develop several obstetrics complications like gestational hypertension, preeclampsia, miscarriage and ectopic pregnancy. Present epidemiological data shows approximately 20-30% of women continue to smoke during

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pregnancy which makes it a major health issue. Pregnancy is a physiological state with enhanced metabolism and demand for oxygen. In addition, active smokers during pregnancy get exposed to more than 4 800 harmful compounds present in the particulate and vapor phases of cigarette smoke which causes macromolecular damages. Red Blood cells (RBCs) contain numerous sources of oxidants with well-equipped redox buffering system. Till now RBCs were considered as a sink for nitric oxide (NO) derived from vascular endothelial nitric oxide synthase (NOS3) and limits the bioavailable NO for vasodilation. Recent evidence showed RBCs to have a new "Erythrocrine function" that possess a functional NOS (NOS3 like) which produce and release bioactive NO and can regulate vascular homeostasis. RBCs can mediate hypoxic vasodilation due to decreased oxygen saturation by exporting NO bioactivity. Sustained smoking during pregnancy, lowers oxygen levels, increases the concentrations of transition metal ions that release reactive oxygen species and intervenes with RBCs. The study evaluated the redox state of the RBCs with its morphological, rheological and functional alterations in the heavy smoking pregnant adults compared to control pregnant adults. It showed distinct morphological variations, significant changes in the post translational modification of NOS3, macromolecular damages developed by 4-hydroxy-2-trans-nonenal staining, a product of lipid peroxidation and rheological alterations as indicated by atomic force microscopy. Thereby, smoking during pregnancy causes irreversible changes that leads to macromolecular damages which induces adverse outcomes in the gestation period.

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Gut region-specific alterations of the endogenous heme oxygenase system and pro-inflammatory cytokines in the enteric neurons of streptozotocin-induced diabetic rat model

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Increase of the hyperglycaemia-induced oxidative stress and decreased effectiveness of the endogenous antioxidant enzymes plays a major role in the initiation of diabetes-related neuronal damage. Nitrergic myenteric neurons in the different gut segments displayed different susceptibilities to diabetes and insulin treatment. Therefore, we aimed to study the gut segment-specific differences in the expression of heme oxygenase (HO) isoforms and their co-localization with neuronal nitric oxide synthase (nNOS) in myenteric neurons as well as the proportion of HO1-immunoreactive (IR), HO2-IR and nNOS-IR submucous neurons in a type 1 diabetic rat model. We also attempted to reveal the gut segment-specific differences in the expression of tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6) in the myenteric ganglia and its microenvironment in type 1 diabetes. Ten weeks after the onset of hyperglycemia, segments from duodenum, ileum and colon of streptozotocin-induced diabetic, insulin-treated diabetic, and control rats were processed for double-labelling fluorescent immunohistochemistry, post-embedding electron microscopic immunocytochemistry and enzyme-linked immunosorbent assay. The number of HO-IR and nNOS-HO-IR myenteric neurons were significantly increased in the diabetic ileum and colon. The proportion of nNOS-IR and HO-IR submucous neurons were highly pronounced in the distal parts of gut in diabetic and insulin-treated diabetic rats. The expression of TNFα and IL6 were strictly gut region-dependent in the myenteric ganglia and supplying capillary endothelium in controls, diabetic and insulin-treated diabetic rats. Based on these results, we suggest that the regional differences in the induction of the endogenous HO system as well as the investigated pro-inflammatory cytokines are strongly correlated with diabetes-related region-specific nitrergic neuropathy.

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Structural elucidation of bioactive peptaibols and understanding their dynamics

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Amongst the plethora of secondary metabolites produced by fungi from the genus *Trichoderma*, peptaibols deserve special attention

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due to their demonstrated anti-bacterial, anti-fungal, anti-viral and anti-helminth properties. These peptides have an acetylated N-terminal and a C-terminal 1, 2-amino alcohol. Non-standard amino acid residues like D-isovaline (Div) and the highly studied residue aminoisobutyric acid (Aib) also constitute their primary structure. They are known to aggregate and form pores across bilayer membranes. The knowledge of their three-dimensional structures and dynamics of channel forming is therefore crucial for understanding their bioactivities.

The non-standard residues of *Trichoderma* peptaibols were first parameterized using RESP charges. Various paracelsins, hypomurocins and peptides from the recently described tripleurin class were simulated under different solvents, starting structures and time periods. The longer peptides like paracelsins show higher propensity to form continuous helix formation. The number of Aib residues in the sequence promotes helicity in the sequence which may fluctuate between left- and right-handedness depending on the neighboring amino acid residues. New tripleurins were seen to conform into a linear helical shape with beta-bend ribbon spirals at the N-terminal and a 3_{10}/alpha-helix at the C-terminal. Peptide folding in methanol solvent promotes stability of the secondary structure more than aqueous solvent. These peptides show a characteristic break from helix continuity at the Aib-Pro bond which confers hinge-like movement to the C-terminal. This movement probably plays a crucial role in channel arrangement.

These results will be used further to model ion channels and visualize their interaction with the membrane. The study of exact mechanism of channel formation and ion expulsion is in the pipeline.

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High-throughput screening to identify inhibitors of PCNA ubiquitination

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Cancer is a major disease with a high rate of mortality. According to the worldwide cancer incidence statistics published by Cancer Research UK, there were 14.1 million new cancer patients in 2012 and there will be 23.6 million new cancer cases per year by 2030. In cancer research, major challenges are in early diagnosis and developing highly effective treatments with low toxicity. DNA replication in cells can be stalled by DNA damage, requiring repair through translesion synthesis (TLS), which is an error-prone pathway and can lead to mutations that favor cancer cell growth and metastasis, as well as result in DNA damage tolerance and therapeutic resistance to numerous anticancer agents.

PCNA is a homotrimeric protein complex that serves as a sliding clamp during DNA replication and as a co-factor for TLS polymerases. When replicative DNA polymerases encounter stretches of damaged DNA, the replication fork stalls. In response, PCNA undergoes monoubiquitination on a specific lysine residue, activating the mutagenic TLS pathway. We are interested in discovering small molecules that inhibit the monoubiquitation of PCNA, the trigger for TLS and so downstream interactions of PCNA with TLS polymerases such as Pol eta (η). This process is central to DNA damage tolerance. Pol η-PCNA complexes have been demonstrated in yeast cells by using a novel genetic code expansion system.

We have developed a functional *in vitro* ubiquitination assay that displays virtually 100% PCNA ubiquitination in control samples and have scaled the assay to high-throughput levels. Screening of NCI/DTP Diversity Set IV, Mechanistic Set IV, Approved Oncology Drugs Set VIII, and Natural Products Set IV with AlphaScreen/AlphaLISA system is underway.

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The redundant role of formins during pigment cells development in the *Drosophila* eye

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The highly dynamic actin cytoskeleton is one of the structurally and functionally most important cellular constituents. The dynamic assembly and disassembly of the actin filaments is controlled by a large set of regulatory proteins some of which also contribute to the formation of higher-order actin structures, such as bundles and networks of filaments. One of the most important classes of the actin regulatory proteins are designated as actin nucleation factors that promote the formation of new actin filaments. Among the three major types of actin nucleation factor that have so far been identified, we focus our studies on the formin protein family.

Formins are highly conserved cytoskeleton regulatory proteins that act in dimers and support the assembly and elongation of new actin filaments. The major aim of my studies is to examine the potentially redundant role of two formins, DAAM and FRL, during eye development in *Drosophila melanogaster*. We have previously shown that these two actin assembly factors play redundant roles during axonal growth in the mushroom bodies of the adult brain. We also noticed that although dDAAM and FRL are strongly expressed in the developing eye and in axons of the photoreceptor cells, *dDAAM* or *frl* single mutant animals do not exhibit any developmental abnormalities in the eye. On the contrary, *dDAAM; frl* double mutant adult flies exhibit rough eyes and we observed a unique and novel eye phenotype: the pigment cells (also known as interommatidial cells) are often detached from the basal lamina and fail to undergo a typical cell shape change required to properly seal the bottom of the unit eyes. These results suggested that these formins act redundantly during eye development. To determine the cellular role of these two formins, we are currently analysing the changes in cell shape and cytoskeleton organization during eye development in the double mutants, whereas the single mutants are used as controls.

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Functional Analysis of a novel hydrophobic surface binding protein in Mucorales

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Hydrophobic surface binding protein A is a small secreted protein found in eukaryotes. This protein was firstly isolated from *Aspergillus oryzae* culture broth. That protein was found to be able to recruit cutinase 1 (Cult1) to the surface of hydrophobic solid materials and could promote the activity of degradative extracellular enzymes. This protein also participates in fungal resistance to stress that could be caused due to toxicity of some aromatic compound or reactive oxygen species released during the degradation process. During infection of MH-S macrophages, *Lichtheimia corymbifera* expressed an Hsb-A-like protein at high level. Hsb-A protein is functionally uncharacterized in Mucorales and its role in the host-pathogen interactions is yet unknown. The objective of the current study was to characterize Hsb-A proteins and the encoding genes of *Mucor circinelloides*.

We found six *hsb-A* genes in the *M. circinelloides* genome, which are homologous to the *L. corymbifera hsb-A*. Two genes (*hsb-A1* and *hsb-A2*) were found to be highly expressed during the life cycle of the fungus. Hence, these two genes were used for further studies. To analyse the possible role of Hsb-A1 and Hsb-A2 in the pathogenesis of *M. circinelloides*, deletion and overexpression mutants were constructed. For overexpression, the genes were placed under the regulation of the strong *gpd1* promoter. To create *hsb-A* knock out mutants, a recently developed CRISPR-Cas9 system was used. Micro- and macromorphology assays were conducted for the deletion and overexpression mutants. Host-pathogen interaction assay was conducted using *Drosophila* models. Furthermore, *Pichia* expression systems were constructed and the expressed hsb-A1 and hsb-A2 proteins were purified for further analysis.

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Endoplasmic reticulum stress: major player in size-dependent inhibition of P-glycoprotein by silver nanoparticles in multidrug-resistant breast cancer cells

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Multidrug-resistant (MDR) cancer is strongly associated with P-glycoprotein (Pgp) overexpression and has been a major deterrent to current chemotherapeutic regimens, thus demanding novel approaches to defeat resistance. Silver nanoparticles (AgNPs) have gained significant attention in nanomedicine, owing to their multifaceted biological properties, which largely depend on their composition, shape and size. In this study we examined how would a variation in nanoparticle size affect the MDR phenotype of drug-resistant cancer cells. For this purpose, AgNPs of two different sizes (5 nm and 75 nm) were tested on Pgp overexpressing MCF7/KCR as well as on drug-sensitive MCF7 breast cancer cells. Exposures to 75 nm AgNPs significantly inhibited Pgp efflux activity, whereas treatments with 5 nm AgNP did not affect Pgp function. 75 nm AgNPs were less cytotoxic and generated less reactive radicals compared to 5 nm AgNPs. Importantly, large AgNPs potentiated significantly the cytotoxic and apoptotic activities of doxorubicin. In order to explain the molecular mechanisms underlying Pgp inhibition we investigated Pgp expression levels and endoplasmic reticulum stress following AgNP exposures. We did not observe any changes in Pgp expression upon 5 nm or 75 nm AgNP treatments, however larger AgNPs induced notable ER stress in drug-resistant breast cancer cells. The manifested ER stress, characterized by transcriptional and translational activation of ER stress markers and autophagy, proved to be independent from oxidative stress, however correlated well with depleted ER calcium stores. The results infer that 75 nm AgNPs are more effective than 5 nm AgNPs in Pgp inhibition due to the entrapment of unfolded or misfolded Pgp in the endoplasmic reticulum as a consequence of ER stress. This mechanism also explains the sensitizing effect of 75 nm AgNPs on drug-resistant MCF7/KCR cells to doxorubicin induced apoptosis.

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Characterisation of Plagl1, a putative downstream target of Rybp

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Congenital heart disorders (CHD) arise from mutations causing structural and functional anomalies in the developing heart. Embryonic stem (ES) cell based *in vitro* differentiation assays are powerful and unique model systems to investigate the underlying molecular events of normal heart development and CHD conditions.

We have previously shown that ES cells, which are lacking the polycomb Ring1 & YY1 binding protein ($rybp^{-/-}$) could not form beating cardiomyocytes *in vitro*. Importantly, expression of several key cardiac transcription factors was strongly affected in the $rybp^{-/-}$ cultures in comparison to the wild-type ($rybp^{+/+}$) counterparts. One of the most affected gene was the Pleiomorphic adenoma gene like 1 (Plagl1), which was nearly absent in the $rybp^{-/-}$ ES cells. Plagl1 is a key cardiac trancription factor: gene targeting experiments has proven that mice lacking Plagl1 develop arterial and ventricular septum defects with thin ventricular wall formation.

During my PhD work, I have further characterised the Plagl1 gene and established its possible relationship with Rybp. Our findings showed that Plagl1 has a complex genomic locus containing three promoter regions coding at least two isoforms of Plagl1 and two non-coding RNAs (ncRNAs) Hymai & Plagl1 I.T. Gene expression analysis of Hymai and Plagl1 I.T revealed that the mRNA levels of these two ncRNAs were greatly affected in the $rybp^{-/-}$ cells during the time course of *in vitro* cardiac differentiation in comparison to the $rybp^{+/+}$ cultures. I have also shown that Plagl1 protein is mostly abundant in the late phase of, *in vitro* cardiac differentiation. Finally, by using *in vivo* luciferase promoter assays, I demonstrated that Rybp was able to activate the Plagl1 promoter through its P3 promoter.

These data suggests that Rybp may exert its functions partially via by activating Plagl1. Considering that Rybp is a repressor protein, current work broadens our limited knowledge on how repressor complex members may also exert their functions as activators during healthy development or disease conditions, like CHD.

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Systematic chemogenomic analysis reveals diverse genetic modulators of bacterial susceptibility to antimicrobial peptides

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Antimicrobial peptides (AMPs) are immune effectors and promising therapeutic agents with diverse mechanisms of bacteria killing. However, a comprehensive understanding of the genetic factors that influence bacterial susceptibility to AMPs is prerequisite to ensure their clinical use and to minimise possible cross-resistance to our immunity peptides.

Here, we performed a chemogenomic analysis to systematically assess the impact of all single genes overexpression on *Escherichia coli* susceptibility to 15 different AMPs, including 2 major types of human host defense peptides. We found that multiple genetic determinants influence bacterial resistance and notably, these resistant determinants largely differ to AMPs with different modes of action. Chemogenomic profiles classified the AMPs according to their physicochemical similarities and outlined their broad modes of action. We demonstrated that human AMPs had a very similar chemogenomic profile to a specific class of membrane disruptive AMPs but largely dissimilar to intracellular targeting AMPs. These differences between AMPs facilitated the identification of a compendium of genes showing collateral sensitivity interactions between the AMPs. We confirmed these findings through a laboratory evolutionary experiment and showed that bacterial adaption to human beta-defensin-3 results in cross-resistance to the membrane disruptive AMPs, but collateral sensitivity to intracellular targeting AMPs. Functional analysis of the gene sets that underlie this collateral sensitivity interaction highlighted a clinically relevant molecular pathway that controls outer membrane asymmetry. Overall, these findings have important implications for the therapeutic development of AMPs.

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Functional characterization of RLCK VI_A kinases with usage of a miRNA-induced gene silencing system

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The Rho-of-plants (ROP) G-proteins are involved in regulation of cell growth, cell polarity, hormonal and pathogen responses but downstream components of ROP mediated signalling are poorly known. A small subgroup of plant specific receptor-like cytoplasmic kinases (RLCK VI_A) shows ROP-binding-dependent in vitro kinase activity. Our aim was to characterize the function of RLCK VI_A kinases in *Arabidopsis* plants.

In order, to investigate the effects of the loss of RLCK VI_A kinase function, we used a trans-acting micro-RNA-induced gene silencing system (MIGS). This system uses a short (22 nc) *Arabidopsis* specific miRNA (miR173) recognition fragment, which can trigger the production of trans-acting small interfering RNAs (tasiRNA) of adjacent sequences. We fused RLCK VI_A1 and A2 kinase specific gene fragments upstream to miR173 recognition sites in tandem organisation. Agrobacterium mediated plant transformation of Col-0 wild type and *rlck vl_a3* T-DNA insertional mutant plants were performed with the MIGS construct.

The silenced gene expression resulted reduction of rosette size and alteration in the phyllotactic pattern of shoots. Pollen tubes of the transgenic plants also exhibited altered growth characteristics. We found that reduced expression of A1, A2 and A3 kinases caused branching and abnormal pollen tube growth. These results indicate the potential role of the kinases in plant growth and development as well as pollen tube polarity in agreement with their expression pattern.

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Analysis of autophagy-related (*Atg*) genes in *Drosophila melanogaster*

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Autophagy is a conserved intracellular degradation process in eukaryotic organisms. This catabolic pathway plays a very important role in cells: the continuous turnover of macromolecules and cell organelles is important for longevity, and it is essential in adaptation to nutrient-poor conditions during starvation. As the autophagy-related (Atg) genes were first described only about 26 years ago in yeast, we still have only limited information about their functions. Via molecular genetic, cell and developmental biology investigations of the *Drosophila* autophagy-related genes (*Atg*), we can clarify their roles.

During my work, I created null mutant alleles for the *Drosophila Atg5*, *Atg9*, *Atg14* and *Atg8b* genes using the CRISPR-CAS9 method and, also for the *Atg8a* gene by gene trapping. These mutants were used both in our own research projects and in an international collaboration as well. The main focus of my work is the investigation of *Atg8* genes in *Drosophila*. Atg8 is an ubiquitin-like protein that is conjugated to the lipid phosphatidylethanolamine (PE) on the phagophore membrane during autophagy. This process is mediated by factors analogous to the ubiquitylation reaction: the Atg8 conjugation machinery also includes factors with E1, E2, and E3-like functions. Only two Atg8 homologs are found in *Drosophila*: the *Atg8a* and *Atg8b* genes. To generate a null mutant allele of *Atg8a* we modified an intronic Minos element of the *Atg8a* locus by inserting a Trojan-Gal4 cassette, which is designed to function as a gene trap. During the characterization of this new allele - based on anti-Atg8 and anti-p62 western blots and somatic mutant clones in the larval fat body - we proved that the created allele functions as an *Atg8a* null mutant, and all homozygotes die during the pharate adult stage. Additionally, we used CRISPR gene editing to generate a nonsense mutant *Atg8a* allele encoding a truncated (G116stop) protein defective in PE conjugation. Animals expressing this non-lipidatable Atg8a are autophagy defective, but viable and fertile. As for *Atg8b*, it is not required for autophagy, and animals homozygous for this allele are adult viable and female fertile but nearly completely male sterile. *Atg8b* mutant sperm cells can develop until the latest stages of spermatogenesis but they are immobile, which explain the male sterility.

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Investigating the role of Wnt/PCP proteins in axon growth and the regulation of neuronal cytoskeleton

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In *Drosophila melanogaster* there are 6, evolutionary highly conserved, core Planar Cell Polarity (PCP) proteins that are essential in the formation of planar polarity in epithelial tissues. Besides this function, these proteins are also known to play multiple roles in neuronal development. Neurons are highly polarised cells with extended neuronal protrusions, during the growth and maintenance of which the neuronal cytoskeleton plays a crucial role. The aim of my project is to study how the core PCP proteins regulate the neuronal cytoskeleton, and to understand how they contribute to axon growth in *Drosophila melanogaster*.

To this end two strategies were applied: I used primary embryonic cell cultures which provide powerful subcellular readouts to determine the state of the neuronal cytoskeleton; in addition, I studied the embryonic and the adult central nervous system in order, to assess the *in vivo* relevance of my findings.

My results showed that, the loss of some PCP components affected axon growth in the embryonic central nervous system. This was in accordance with my findings in primary neuronal cell cultures. Furthermore, I found that the lack of PCP components led to changes in microtubule (MT) organisation in cultured neurons. In order, to investigate the mechanism by which PCP proteins can regulate the MT cytoskeleton I also studied Daam, a formin type protein which is a known cytoskeleton regulator and has been linked to PCP signalling previously. In the absence of Daam MT organisation was strongly affected, moreover, I found that Daam has an important role in regulating MT dynamics and stability as well. Further ongoing experiments show that PCP proteins, as well as Daam, control

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axon growth and guidance in the ventral lateral neurons (LNv) of the adult brain. Together these findings suggest that PCP proteins are necessary to properly regulate MTs, presumably by controlling Daam, and contribute to axon growth *in vivo*.

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The anti-amyloidogenic effect of natural product extracts on amyloid-like fibril formation of trypsin in aqueous organic solvents

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The formation of amyloid fibrils has been associated with several human diseases. The appearance of amyloid aggregation is an indicator of different central nervous system neurological disorders and neurodevelopmental diseases, which affect the brain and peripheral tissues. The misfolding and aggregation of proteins cause a large number of different neurodegenerative diseases. Amyloid is a generic structural form of the polypeptide chain and most proteins can form amyloid-like fibrils under proper conditions.

Natural product extracts contain important bioactive compounds without undesirable side effects, which are necessary for the prevention and cure of various diseases. Fifty-two phenolic compounds were identified in culinary herbs and spices. The aromatic rings of polyphenols may competitively interact with aromatic residues in amyloidogenic proteins, prevent the π-π interaction and block the self-assembly process. The phenolic hydroxyls of polyphenols may inhibit amyloid fibril formation via binding the hydrophobic residues in amyloidogenic proteins. Here we report the inhibitory effect of some natural product extracts on the formation of amyloid fibrils using trypsin as a model protein, in aqueous ethanol. Inhibition of aggregation and fibrillation of trypsin was determined based on turbidity measurement, aggregation kinetics assay, amyloid specific dye Congo red (CR), fourier-transformed infrared (FTIR) spectroscopy, electronic circular dicroism (ECD) and transmission electron microscopy (TEM). The experiments revealed that great anti-fibrillation activity was exerted by chili extract, *P. ginseng* extract, grapefruit seed extract, peppermint extract, Eduscho coffee extract and Egri bikavér red wine. It was found that the amount of fibril formation was greatly reduced with increasing concentration of extracts and the inhibitory effect is dose dependent.

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Studies on the sexual development of *Aspergillus nidulans*

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Studies on the sexual development of *Aspergillus nidulans* revealed more than 50 regulatory elements involved in the activation or repression of sexual development. In our previous studies we had identified three, architectural chromatin associated HMGB proteins in *A. nidulans* (HmbA, HmbB and HmbC) and developed deletion mutants. We noted that viability of ascospores is dramatically reduced in *hmbBΔ* and *hmbAΔ* and *hmbCΔ* mutants are self-infertile. Accordingly, we suppose that HMGB proteins play crucial role in the sexual development at chromatin level. We aimed to study the sexual development in the deleted mutants and compare to that of wild type. Through the microsopic analysis of the sexual structures we characterized the sexual impairments of the *hmbAΔ*, *hmbBΔ* and *hmbCΔ* strains. In order, to shed light on the molecular basis of the mutant phenotypes and evaluate the governing role of HmbA/B/C proteins on sexual development, we carried out gene expression analysis on 36 transcription factors involved in sexual development.

A. nidulans produces the carcinogenic sterigmatocystin (STC) toxin, which is thought to be a protective compound for the protection of the fungal reproductive structures. Studies on the sexual development and secondary metabolite biosynthesis have revealed the association of these two processes in time at regulatory level. We aimed to study the spatial distribution of STC production within the colony and assess its association with sexual development by employing a yCFP reporter system. We developed an *stcO* reporter strain by substituting the ORF of *stcO* with nucleus targeted yCFP. We proved that *stcO* has essential role in STC biosynthesis and

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activation of gene expression is restricted to the third day of incubation. We demonstrated that the *stcO* promoter is active only in vegetative hyphae that surround groups of hülle cells and the activity decreases and eventually ceases as the distance between the hypha and the hülle cells increases. This phenomenon indicates that the vegetative mycelium might consist of morphologically uniform, but functionally different hyphae.

This work was supported by the Hungarian Government and the European Union within the frames of the Széchenyi 2020 Programme through grant GINOP-2.3.2-15-2016-00012. The the infrastructural background was established with the support of GINOP-2.3.3-15-2016-00006 grant (Széchenyi 2020 Programme).

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Dynamic gene duplication/loss history marks the unique evolutionary route to fungal multicellularity

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Multicellularity has evolved numerous times during eukaryote evolution, yet the genetic prerequisites for these transitions are hardly known. In contrast to other organisms fungi used their own unique evolutionary route to achieve multicellularity with different physiological bases. This raises the question whether the genetic-mechanistic principles of the evolution of multicellularity are common to both fungi and animals and how fungal multicellularity-related gene families evolved during the history of life. Here we reconstruct the evolution of the genetic background of fungal multicellularity based on both known multicellularity-related genes from the literature and genome-wide identification of gene families that evolve in a correlated fashion with multicellularity. Based on literature surveys, we identified 493 genes involved in the establishment and maintenance of cell polarity, vesicular transport and cytoskeletal rearrangement. The evolutionary origins of these genes were examined using complete genomes of 71 unicellular and multicellular eukaryotes. We implemented phylostratigraphic analyses using a custom pipeline, which uncovered the evolutionary origins of multicellularity-related genes, and reconstructed gene duplication and loss histories by COMPARE analysis. This study yielded a high-resolution view of the dynamics of these known fungal multicellularity-related gene families. Further we could identify 316 gene families, including certain cytochrome P450 families, monocarboxylate permeases and vacuolar aspartyl proteases that show strong correlated evolution with multicellularity, providing candidates for future functional studies. Our results indicate that part of the genetic toolkit behind fungal multicellularity was already present in ancestral unicellulars and that some of the hyphal morphogenesis related gene families show diversification before the emergence of the first filamentous fungi. These results would suggest that beside the *de novo* gene family birth and gene duplication events, hitherto unknown gene regulatory mechanisms could also have had a crucial role in the evolution of multicellular fungi.

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Characterization of lytic bacteriophages against biofilm-forming multi drug resistance *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a most common opportunistic multi-drug-resistant (MDR) pathogen that is poised to become a widespread problem. Recently some strains even show additional evolved resistance to 'drugs of last resort', resulting in emergent strains that are pan-drug-resistant (PDR). The bacterium has high endogenous resistance to many antibiotics, because of its outer membrane barrier, multidrug efflux pumps, endogenous antibiotic inactivation and biofilm-formation. Biofilm mediated infections including catheter-associated urinary tract infections and ventilator-associated pneumonia. One alternative treatment for MDR bacterial infections is phage therapy: the use of lytic bacteriophages as in situ self-amplifying 'drugs' that specifically target and kill bacteria. Phages also have

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several properties allowing them to act on biofilms. They might produce enzymes that disintegrate the extracellular matrix. The high numbers of bacteria in the biofilms facilitate the action of phages by allowing rapid and efficient infection. In my study, I focused on *P. aeruginosa*, more than Twenty strains belonging various serotypes and 13 lytic phages were isolated and characterized. The morphology of phages was analyzed by electron microscopy, the genomes of the phages and few hosts were studied by NGS technologies. The serotype and the host specificity of the phages were studied in liquid cultures and on biofilms. A major limitation of phage therapy is the potentially narrow host range of the phages; a specific phage is often capable of lysing only one or a small number of strains within a species. To overcome this limitation, we used phage cocktails and also performed co-cultured study harboring phage-resistant and sensitive bacteria. The effectivity of phage cocktails on recalcitrant biofilm architecture was examined by confocal laser scanning microscope. A defined cocktail composed of 5 phages with distinct serotype specificity was effective against any biofilm-formers which clearly indicates the applicability of phages against *P. aeruginosa* infections.

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The role of chloramphenicol in enhancing photodamage of Photosystem II in *Synechocystis* 6803

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Photoinhibition is light induced reduction of photosynthetic capacity in plants algae, and cyanobacteria. Light damages primarily the structure and function of the PSII complex, and the damage is repaired by the re-synthesis of the D1 protein subunit. The rate of photodamage can be monitored in the presence of photosynthetic inhibitors like lincomycin and chloramphenicol, which block the repair. It has been reported earlier that chloramphenicol serves as an electron acceptor of PSI and its reduction intermediate transfers the electrons to molecular oxygen to produce superoxide. We have studied the effect of chloramphenicol on the rate of photodamage in isolated PSII membrane particles and in *Synechocystis* 6803 cells. By using the isolated PSII membranes we have demonstrated that chloramphenicol can accept electrons and mediate superoxide production not only in PSI but also in PSII. We have also shown that the chloramphenicol mediated superoxide production is responsible for accelerated photodamage of PSII in isolated PSII membrane particles.

We have also studied the effect of chloramphenicol on the rate of photoinhibition, as well as on superoxide production in intact *Synechocystis* 6803 cells. In order, to obtain the net rate of photodamage without the effect of the ongoing repair of PSII the light treatment was performed in the presence protein synthesis inhibitors, either chloramphenicol or lincomycin. Our results showed that the rate of photodamage was enhanced in the presence of chloramphenicol as compared to that in the presence of lincomycin. By using oxygen uptake measurements in the presence and absence of superoxide dismutase (SOD) we could show that chloramphenicol mediates the production of superoxide in *Synechocystis* 6803 cells which only PSII complexes and lack PSI. These data indicate that superoxide, which is produced via interaction of chlorampehicol with PSI and PSII in WT cell, or with PSII in the PSI-less mutant *Synechocystis* 6803 is responsible for the enhanced photodamage.

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Development of targeted DNA methylation tools for breast cancer research

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In estrogen receptor alpha positive (ER+) breast cancer anti-estrogen endocrine therapy is a standard treatment. Unfortunately, many initially responsive patients develop resistance to the therapy. There are data suggesting that epigenetic changes such as DNA methylation and histone modifications play crucial roles in endocrine resistance. As a member of a Marie Skłodowska-Curie Innovative

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Training Network focusing on epigenetic regulation of endocrine therapy resistance in breast cancer, we develop research tools for targeted DNA methylation. Our goal is to identify genes which have a role in resistance acquisition.

We have constructed a CRISPR/dCas9-guided DNA methyltransferase (MTase) tool set by creating genetic fusions between the catalytically deficient dCas9 protein and the CpG-specific prokaryotic C5-MTase M.SssI. To improve the specificity of methylation, we used mutant forms of M.SssI. Initial testing of the targetable chimeric MTases in *E. coli* indicated that the fusion construct involving the Q147L mutant of M.SssI provided the highest specificity. Targeted DNA methylation experiments in cultured breast cancer cells were performed in collaboration with M. G. Rots' laboratory at Groningen University. Promoter regions of the HesI, YYI, SLC9A3R1 and CD44 genes implicated in endocrine therapy resistance were targeted by transiently expressing the dCas9-MSssI protein. Analysis of gene expression by qPCR showed that CD44 expression could be repressed by methylation of the CD44 promoter. To be able to select transfected cells by cell sorting, a plasmid expressing the dCas9-M.SssI-P2A-mCherry fluorescent fusion protein was constructed.

We have constructed plasmid vectors and developed a method facilitating combination of approaches involving transient expression as well as stable expression of epigenetic effectors in mammalian cells.

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Functional characterization of the mevalonate-isoprenoid biosynthesis pathway genes in *Mucor circinelloides*

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Members of the subphylum Mucoromycotina, order Mucorales (such as *Lichtheimia, Mucor, Rhizomucor* and *Rhizopus* species) are saprotrophic fungi, which also have industrial and agricultural importance. Several species belonging to this fungal group are also considered to opportunistic pathogens, which can cause fatal systemic infections (so-called mucormycosis) in immunocompromised patients.

Metabolites synthesised via the mevalonate-isoprenoid pathway (such as sterols, functional groups of proteins and carotenoids) play an important role in signal transduction, morphogenesis, adaptation to environmental change and protection against free radicals. Nowadays ergosterol and its biosynthesis is the major target of the antifungal agents used in clinics. The therapy of mucormycosis is still limited because of the intrinsic resistance of these fungi to the majority of the currently used antimycotics. To date little is known about the function and regulation of the mevalonate-isoprenoid biosynthesis pathway genes in Mucoromycotina fungi. Our aim was to characterize six genes of that pathway in *Mucor circinelloides*, encoding the HMG-CoA synthase (*hmgS*), mevalonate kinase (*mvk*), diphosphomevalonate decarboxylase (*dmd*), isopentenyl pyrophosphate isomerase (*ipi*), farnesyl pyrophosphate synthase (*isoA*) and geranylgeranyl pyrophosphate synthase (*carG*).

Effect of different cultivation conditions on the gene transcriptions was analyzed. Plasmids were constructed for silencing and overexpression of the genes and the micromorphology of the mutants was analyzed. Silencing of the mevalonate-isoprenoid genes led to significant reduction in the ergosterol content in comparison with the wild-type strain, furthermore increased carotenoid content was determined in the transformants harbouring the *mvk*, *ipi* or *carG* gene in extra copies. Overexpression and silencing of the *hmgS*, *dmd* and *ipi* genes led to significant change in the susceptibility to azoles and statins. Phagocytosis assay with the mutants and MH-S macrophages was also performed.

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A novel pathophysiological evidence for HCN channels in absence seizures and role of brain pericytes in inflammatory responses induced by bacterial-derived agents *in vivo*

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and the I_h current they generate contribute to the pathophysiological mechanisms of absence seizures (ASs), but their precise role in thalamocortical circuit, the main components of the network underlying AS generation remains controversial. Thus, we showed that the pharmacological block of HCN channels with the antagonist ZD7288 applied via reverse microdialysis in the ventrobasal thalamus (VB) of freely moving GAERS decreases TC neuron firing and abolishes spontaneous ASs. Moreover, thalamic knockdown of HCN channels via virally-delivered shRNA into the VB of Stargazer mice decreases spontaneous ASs and I_h-dependent electrophysiological properties of TC neurons. These findings provide the first evidence that block of TC neuron HCN channels prevents ASs and suggest that any anti-absence therapy that targets HCN channels should consider the opposite role for cortical and thalamic I_h in the modulation of ASs. Cerebral pericytes are perivascular cells involved in the formation and control of the neurovascular unit (NVU). Growing evidence suggests that they also play definitive role in neuroinflammation. Among the pattern recognition receptors common to the innate immune system, we previously detected *in vitro* expression of several NOD-like receptors both basally and, in response to inflammatory mediators. Upon inflammatory stimuli, these receptors with other inflammasome components recruit and activate caspases via canonical or non-canonical routes, resulting in cleavage of precursor cytokines or pyroptosis. To assess the inflammasome activation *in vivo*, either outer membrane vesicles (OMVs) isolated from *E. coli* bacteria or lipopolysaccharide are administered into the common carotid artery of transgenic mice that express red fluorescent protein (DsRed) in perivascular cells. Two-photon-assisted imaging and immunohistochemical stainings are in progress to elucidate if inflammasome activation can induce changes in the function of the NVU after such an inflammatory challenge.

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Processes of selenium toxicity in different plant species

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Selenium is a non-metal element essential to all living organisms except higher plants. Accumulated selenium is able to damage plants *via* toxic processes, such as: disturbances in carbon and nutrient metabolism, disturbance in hormonal homeostasis, non-specific seleno-amino acid production and nitro-oxidative stress. Nitro-oxidative stress consists of abnormal ROS and RNS homeostasis and changes to macromolecules *via* nitration or nitrosilation. I studied protein tyrosine nitration, which is irreversible in plants and most likely inactivates the protein. The experiments were carried out on plant species with different selenium tolerance. Selenium sensitive Arabidopsis (*Arabidopsis thaliana*) was compared with selenium tolerant indian mustard (*Brassica juncea*); a selenium sensitive medicinal plant, *Astragalus membranaceus* was compared to a selenium hyperaccumulator *Astragalus bisulcatus*. The research was conducted in three different experimental designs and the treatment was with sodium selenite and selenate. We measured total selenium content, morphology, viability, ROS and RNS levels and protein tyrosine nitration.

In all experiments plants have accumulated selenium, which changed the morphology of sensitive plants. Protein tyrosine nitration was most significant in *Astragalus membranaceus* compared to all other plant species, describing a heavy stress and a reduction of the active protein pool. In the experiments where different selenium forms were compared, selenite was more toxic compared to selenate.

According to our data there is a strong correlation between selenium sensitivity and protein tyrosine nitration. The tolerant plants

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species can cope better with protein tyrosine nitration compared to the sensitive species, where the nitration is more significant.

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Brain state dependent activity of thalamocortical cells

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The mammalian brain exhibits various brain states including focused attention, relaxed wakefulness, sleep etc. These states are associated with altered neural activity in various brain structures and can change on a rapid time scale. Recent results suggest that the activity of the neurons in the somatosensory and the visual cortex correlate with these brain state changes, but whether and how neurons in subcortical regions are involved in this phenomenon has remained elusive. The thalamus relays sensory information to primary sensory cortical areas. In our experiments we focused on the brain state dependent fluctuations of neurons in the visual thalamus, *i.e.* the lateral geniculate nucleus (dLGN) which conveys visual information to the primary visual cortex (V1).

We used electrophysiological methods to record the electrical activity of individual neurons in the dLGN and V1 while simultaneously monitoring the local field potential in the dLGN and V1 and pupil diameter of awake, head restrained mice. Pupil diameter is a well-established indicator of brain states. The pupil of the mouse was recorded using a high frame rate (300 fps) infrared camera and the pupil diameter quantified using custom written routine in ImageJ software. Our results show that the spontaneous electrical activity including extracellular action potential output and the membrane potential of the majority of dLGN neurons is positively correlated with pupil diameter, whereas a minority of neurons negatively correlated and the remainder not correlated. To assess the effects of brain states on thalamic visual information processing we presented moving gratings of 8 different orientations presented on a PC screen 20 cm from the contralateral eye of the mouse. Our data analysis suggests that the orientation tuning of thalamocortical neurons in the dLGN is also varies slightly with brain state dynamics, leading to a further suggestion that brain state modulation also affects visual performance.

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Exploring evolutionary processes by targeted *in vivo* mutagenesis

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Genome engineering has opened a new avenue of research in biology. By constructing mutations orders of magnitude faster than natural evolution genome engineering made the analysis of long-term evolutionary processes feasible within laboratory timescales.

Since the underlying mutations of such processes are rare, efficient exploration of the sequence space requires maximal control over the position and rate of mutagenesis. Specifically, directed evolution of protein complexes and biosynthetic pathways remains a formidable problem, not least because improvement of such traits frequently demands acquisition of multiple mutations simultaneously, many of which provide little or no benefit individually. Technologies enabling targeted mutagenesis of multiple loci in their native genomic context are needed for these goals to be met, however, current *in vivo* mutagenesis methods suffer from serious limitations.

My research addresses the aforementioned limitations of current mutagenesis methods. We developed a technology that enables *in vivo* targeted mutagenesis and precisely generates vast genetic diversity along the full length of multiple, predefined genomic loci.

We demonstrate the potential of this technology by mutagenizing antibiotic resistance genes and comprehensively analyzing

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mutational processes behind antibiotic resistance in multiple bacterial species. We investigate how resistance develops against existing and novel antibiotics and compare these evolutionary processes across pathogens. Moreover, we analyze previously undetected resistance-conferring mutations and generate sensitive structure-activity relationship maps on drug targets.

Finally, we also demonstrate that our targeted mutagenesis technology can be exploited to analyze the mode-of-action of antibacterial drugs in pathogenic bacteria and develop antibiotics that are less likely to suffer from resistance development. Moreover, it is also ideal for biotechnological applications demanding optimization of multiple genomic loci.

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Photoautotrophic H_2 production in *Chlamydomonas reinhardtii*

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The green alga *Chlamydomonas reinhardtii* is one of the most popular organisms used in the research on the photoproduction of H_2. Its hydrogenase enzyme, located in the chloroplast, is highly active, but very sensitive to O_2. H_2 production can be induced by depriving the algae of sulphur, which results in the loss of photosystem II (PSII), responsible for O_2 evolution. However, sulphur deprivation has disadvantages, namely that its effects are unspecific, and it results in irreversible damage and removing sulphur from the culture is a time-consuming process. A less common method for inducing H_2 production is to prepare dense alga cultures, place them in the dark for a few hours under N_2 atmosphere, during which hydrogenases are expressed. When these cultures exposed to light, an initial burst of H_2 production occurs that is subsequently suppressed by O_2 released during photosynthesis. During my PhD studies, we have discovered that continuous and efficient H_2 production lasting for several days can be achieved by keeping the Calvin-Benson cycle inactive by substrate limitation; the protocol we have developed is also fully photoautotrophic, meaning that the electrons used for H_2 production are derived mostly from water.

Our novel H_2 production protocol was also tested on various photosynthetic mutants, including mutants with truncated antenna (*tla3*), reduced chlororespiration (*NDA1*), reduced cyclic electron transport (*pgrl1*), increased amount of PSII reaction center (L159I-N230Y) and reduced state transition (*stt7*). We found that the *pgrl1* mutant produced about approx. 60% more H_2 than the wild-type. A further improvement in H_2 production was achieved also by employing short regeneration phases every 24 hours, which consist of feeding the cultures with CO_2 followed by a dark period. We have also built a photobioreactor with optimized liquid to gas phase ratio and automated gas removal.

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Arabidopsis glutathione peroxidase-like5 (AtGPXL5) is involved in salt stress response and has a function in both the ethylene evolution and polyamine homeostasis

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Plants contain GPX-like (GPXLs) enzymes and are closely related to animal phospholipid hydroperoxide glutathione peroxidases (PHGPX) which play a very important role in protecting against oxidative damage of membranes. Plant GPXLs are regarded to be less important as peroxide scavengers, but it was suggested that they could take part in H_2O_2-based redox regulation. Reactive oxygen species (ROS) and especially H_2O_2 are important compounds of the oxidative stress responses. There are several regulators of ROS homeostasis, among them polyamines (PA) and the plant hormone ethylene. It came to know that the role of ethylene and PAs in signalling processes rather than their accumulation chiefly influences abiotic stress tolerance. Our aim was to investigate the role of AtGPXL5 isoenzyme in response to salt treatment and the ethylene precursor ACC (1-aminocyclopropane -1-carboxylic acid) using *Atgpxl5* T-DNA insertional mutant plants.

The number of lateral roots, length of roots, superoxide level ($O_2^{\cdot -}$), viability and ROS levels were investigated in shoots and roots

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of 12-day-old *Arabidopsis thaliana* Col-0 (wild type) and *Atgpxl5* seedling after one week of treatments using fluorescent microscopy. In another experimental system the wild type and mutant plants grown for 6 weeks in hydroponic system were treated with 1 μM ACC and 100 mM NaCl for 24 hours. Beside measurements of the H_2O_2, malondialdehyde (MDA), ethylene and free PA levels, the activities of diamine oxidase (DAO), polyamine oxidase (PAO) enzymes were determined. The transcript amounts of selected genes were also investigated by RT-qPCR.

The *Atgpxl5* seedlings had more lateral roots even under control conditions, accumulated higher level of ROS and exhibited lower viability than wild type. According to our results, the PA and ethylene amounts changed inversely in mutants after the used treatments, indicating important role of AtGPXL5 protein in regulation of PA and ethylene levels especially under stress conditions.

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Secreted aspartic proteases from *Candida parapsilosis* regulate host complement attack and inflammatory responses

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Candida parapsilosis is an opportunistic fungal pathogen responsible for approximately 30% of the candidaemia episodes in low birth weight infants, while accounts for 10-15% of *Candida* infections in adults. During the infection, fungal secreted aspartic proteases (Saps) play an important role in evading the first line of host defense including the complement responses. However, no detailed studies have been done to investigate the role of Sapps in impairing the host innate immune response including host complement attack. Thereby, in this study, we sought to delineate the exquisite role of Sapps in *C. parapsilosis* mediated pathogenesis. To examine the effect of complement proteins on the growth of the *C. parapsilosis* GA1 (wild-type) and *sapp* $^{-/-}$ strains, their growth was measured in YPD and YCB liquid medium supplemented with 20% of either normal human plasma Data derived from both colony forming unit (CFU) counting and growth kinetics showed that the *sapp* $^{-/-}$ was hypersensitive to the presence of NHP compared to the wild-type. Deletion of *CpSAPPs* neither affects biofilm formation, nor the morphological attributes of *C. parapsilosis*. Virulence properties of both strains were also tested and showed that *sapp* $^{-/-}$ strain induced less damage to human epithelial cells, THP-1 cells and to PBMC-DMs. THP-1 and PBMC-DM's killed and phagocytosed *sapp* $^{-/-}$ cells more efficiently. Furthermore, when examining host cytokine responses, the wild-type strain induced higher levels of IL-1β, TNF-α and IL-8 than the *sapp* $^{-/-}$ strain. We also found that Sapp1p and Sapp2p proteins efficiently inactivate host complement factors such as C3b, C4b and factor H.

In summary, our results indicate that *SAPPs* are an indispensable factor in *C. parapsilosis* mediated virulence.

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Investigation of the archery related skeletal changes in the series of the 10th century AD cemetery of Sárrétudvari-Hízóföld

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In this presentation we introduce the preliminary results of an anthropological investigation of archery-induced stress markers on the skeletons of the Hungarian Conquest Period (10th century AD) cemetery of Sárrétudvari-Hízóföld. According to historical and archaeological data the bow was a common weapon in this era and the Hungarian army based on highly skilled mounted archers. In the Sárrétudvari-Hízóföld cemetery the archery equipment is a frequent type of the grave goods- 58 graves contained arrowheads, quivers

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or bow plates, which suggests that archery was one of the main activities of the concerned population. Our main question is whether anthropological data also reflect this fact, or not. We focused on the so called activity related changes that occur on the skeleton as a result of physical stress, such the entheseal changes, the joint changes, metrical data, traumas and certain types of non-metrical variations. Macroscopic analysis was performed on the bones of the upper limb - the scapulas, claviculas, humeruses, radiuses and ulnas of the "archer" graves and the unarmed adult male graves. Among the different types of activity related changes we found hypertrophy at the attachment of a wide scale of muscles of the upper body and a few of them - such as *m. deltoideus, m. pectoralis major, m. latissimus dorsi, m. brachialis and m. biceps brachii* - appear in high frequency. As a preliminary result we can state that the anthropological and archaeological data do support each other concerning the application of archery in the population in question.

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Characterization of a novel G-quadruplex binding protein

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The basic criterion for staying alive for any living organism is the preservation of the genomic integrity, therefore the greatest challenge in this process is the correct replication of the genetic information during cell division. In order to create an accessible template for the replication process, single-stranded DNA is formed by the unwinding of the parental double-stranded DNA. Since single-stranded DNA sequences often form stable secondary structures, which represent replication blockades, for complete replication, cell division, and for the prevention of apoptosis and cell death these structures must be resolved.

One of the most studied blocking DNA structure is the G-quadruplex (also called G4), which in the recent years has emerged as a key regulatory cis element in essential cellular processes, like transcription, translation, replication and recombination.

The replicative polymerases are primarily blocked by the stable secondary structure-forming DNA sequences, therefore, the cooperation of DNA helicases and DNA polymerases is needed for replication. Some key players of this process are already known, recently it has been described that the mutation rate of G4 sequences increases in the absence of the yeast Pif1 or human FANCJ protein. In order to gain more information about this complex process we searched for uncharacterized players of G4 replication. Here we report the biochemical characterization of a novel G-quadruplex binding protein, which possible function could be the synchronization of the G4 unwinding helicases and DNA polymerases.

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Identification of ascorbate transporters in higher plants and in green algae

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Ascorbate (also called vitamin C) is a multifunctional metabolite in plants. It is an antioxidant and coenzyme for a number of metabolic reactions. It also participates in cellular development, cell wall synthesis and regulation of gene expression.

Ascorbate is produced in the mitochondria and for fulfilling its multiple roles in the cell, it must be transported through several membrane systems. Because of its size and negative charge at physiological pH, ascorbate cannot freely diffuse through membranes. In plants until now, there was only one ascorbate transporter identified, a chloroplastic phosphate transporter, with dual function, called PHT4;4. However, this cannot be the only one chloroplastic ascorbate transporter, because the *pht4;4* knockout mutant does not show any alteration in phenotype.

Our approach to identify chloroplastic ascorbate transporters is based on coexpression analysis using the key ascorbate biosynthesis gene, *vtc2* as a bite, against all the chloroplast-localized transporters. We have found several genes showing relatively high coexpression with *vtc2*. Altogether we screened about 120 T-DNA lines for about 30 transporter proteins, and 5 putative ascorbate transporters were found. We started the characterization of these lines and the initial results confirm that we indeed found novel ascorbate transporters.

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We have also started the molecular characterization of these lines.

We have found 3 homologs (PHT3, PHT4, PHT7) of the PHT4;4 protein in the green alga *Chlamydomonas reinhardtii* and the *PHT3* and *PHT7* genes responded strongly to oxidative stress treatment. In order, to study the function of these proteins, the recently developed CRISPR/Cpf1 genome editing technique was used to generate knockout *PHT3* and *PHT7* lines. The mutant lines show a retarded growth phenotype and decreased photosynthetic performance particularly at high light intensities. In order, to study the cellular localization of these proteins, we started generating YFP constructs.

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Lipid biomarkers - a renewed verification method for the better diagnosis and evaluation of ancient TB infection

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The research of tuberculosis (TB), the pathomechanism and evolution of its infectious agents is surpassingly important since TB is still the 9th leading cause of death. According to the estimation of the WHO 10,4 million people got ill in 2016. TB is caused by the members of the *Mycobacterium tuberculosis* complex, especially by *M. tuberculosis*. Scholars proved that the disease has been presented as a human pathogen at least for 10000 years, but it is widely known since the beginning of the 20th century for its high mortality. The worldwide spread of the TB related problems (e.g. the spread of MDR strains, co-infections) resulted in fast development of the diagnostics. The traditional methods (e.g., acid-fast staining, histology) are going to be replaced in the clinical practice with faster and more effective diagnostic methods which are based on the DNA and lipid biomarker analysis. Their high sensitivity and their target molecules make them suitable to be adapted to osteoarchaeological purposes, and complement the traditionally used macromorphological investigations.

The key elements are the so-called mycolic acids, because they can be preserved in the bones for ages due to their high representation in the lipid rich mycobacterial cell wall, and their structure. The composition of the mycolic acids is supposed to be characteristic to the different species of the Mycobacteria. Our main task was to establish a lipid biomarker based method by the combination and optimization of two existing methods, which allows us to compare the mycolic acid composition of certain Mycobacterial species, in order to differentiate several Mycobacteria and to exclude the false negative results of the environmental *Mycobacteria*. Our main aim is to make comprehensive paleoepidemiological investigations through the examination of bone samples from different archaeological sites.

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Examination of potential epigenetical targets of Huntington's disease in *Drosophila melanogaster*

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Huntington's (HD) disease is a dominantly inherited, late onset, progressive neurodegenerative disorder with characteristic motor, psychiatric and cognitive symptoms. HD is caused by an expansion of a CAG trinucleotide repeat in the first exon of the *huntingtin* gene, which results in an expanded polyglutamine (polyQ) domain in the Huntingtin (Htt) protein. Huntington's disease has a multifaceted molecular pathomechanism that disturbs several cellular processes. Mutant Htt forms intracellular aggregates and participates in abnormal interactions with other proteins. Mutant Htt interacts with histone acetyltransferase enzymes that regulate transcription by modifying lysine (K) residues on histone proteins. These interactions disturb the acetylation balance and lead to transcriptional dysregulation.

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My aim was to characterize the effect of histone acetylation in HD pathogenesis by investigating the influence of the cytosolic acetyltransferase Hat1 and specific histone modifications in a *Drosophila* model of the disease.

To be able to study the role of Hat1 in HD pathogenesis I generated a Hat1 null mutant by P-element remobilization. We found that Hat1 is responsible for the majority of H4K5 and H4K12 acetylation in embryos and influences the transcription of more than 2000 genes, causing a developmental delay. Even though these transcriptional changes, Hat1 mutants are viable and fertile. I found that partial loss of Hat1 moderately ameliorates neurodegeneration in the *Drosophila* HD model. We also studied the effects of histone H4 acetylation marks in the HD model. We generated His4r transgenic lines with substitutions that change specific lysines to glutamine (Q), mimicking acetylated K, or to arginine (R), mimicking not-modified K. Our data show that H4K8Q and H4K16Q substitutions had a positive effect on viability and longevity of the *Drosophila* HD model.

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Analysis of mitochondrial functions in stress response

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Mitochondria, plays central role in the efficient provision of energy for eukaryotic cells by producing adenosine triphosphate and it is also important in production of reactive oxygen species (ROS) which are involved in cellular signalling and stress response in plants. Stabilization of the electron flow in the mitochondrial ETC can protect plants by reducing oxidative damage as well as control of redox balance and support photosynthesis during stress.

Here we describe the *Arabidopsis thaliana cyc1.1* and *cyc1.2* mutants in which T-DNA insertions disrupt the *CYC1-1* and *CYC1-2* genes, respectively. The highly homologous CYC1-1 and CYC1-2 proteins are members of Cytochrome C1 family, and are integral subunits of Complex III, forwarding electrons toward cytochrome c. The two mutants display morphological differences under stress conditions. Phenotype of *cyc1.1* is very similar to the wild type under non-stress conditions. However, under oxidative stress, root growth rate of *cyc1.1* is higher. Measuring rosette size and chlorophyll content also revealed that *cyc1.1* plants are more resistant to oxidative stress. On the other hand, *cyc1.1* is slightly more sensitive to salt stress than *cyc1.2* and wild type. Moreover, *cyc1.2* plants have smaller rosettes and delayed flowering under non-stress conditions and are able to survive for longer time under severe salt stress than wild type or *cyc1.1*. In double mutants with insertions in both *CYC1-1* and *CYC1-2* genes, the electron transport through Complex III presumably strongly reduced, leading to embryo lethality. These observations indicate that CYC proteins have essential function in Complex III.

Although, the studied homologous proteins have very similar structure, our data suggest that they differ from each other in functional properties which influence stress response. Our results suggest that *CYC* genes can be potential targets for engineering in crop plants with aims to improve tolerance to drought or salinity.

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The evolution of complex multicellularity in fungi: from megaphylogenies to single cell transcriptomics

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The Kingdom Fungi is one of the few lineages where complex multicellular structures such as fruiting bodies evolved, however the genetic background of complex multicellularity in fungi is less known. Therefore our aim was to uncover the evolutionary patterns of fruiting body formation and on the genetic toolkit underlying complex multicellularity in fungi.

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Majority of the fruiting body forming fungi can be found in the class Agaricomycetes. By analyzing the variation in diversification rates of this class, we examined whether key innovations and mass extinction events have occurred during the evolution of mushroom forming fungi. To test these hypotheses, the most comprehensive phylogeny of Agaricomycetes was constructed to date. The analyses showed more than 100 shifts in diversification rate, and a putative mass extinction event during the course of evolution. We found that changes in diversification rates of lineages coincide with evolutionary innovations in fruiting body morphology. We found that the evolution of cap could be a key innovation which has contributed to the dominance of agaric mushrooms among extant Agaricomycete species. Hence, knowing the genetic background of fruiting body development would help to understand not only the evolution of complex multicellularity, but also cap formation in fungi, for which we chose an Agaricomycete model organism, *Coprinopsis cinerea*. The transition from single to complex multicellularity takes place when the fungal colony differentiates into a primary hyphal knot. To identify genes that involved into this transition and to examine the differentially expressed mRNA of different cell populations we used Laser-Capture Microdissection (LCM) coupled with single cell RNA Sequencing (scRNA-Seq). We developed a protocol for isolating high-quality RNA from fixed and laser micro-dissected cells and in preliminary scRNA-Seq analyses 50-90% of sequenced read could be mapped into exonic regions. In the future, we will further optimize the scRNA-Seq protocol to reveal the genetic toolkit involved in complex multicellularity and fruiting body development.

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Investigation on secondary metabolites of fungal endophytes from medicinal plants

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Natural sources are always infinite resources of the new molecules for drug discovery as the novel bioactive molecules or as alternative sources of existing ones. Endophytes are the microorganisms residing in the internal tissues of plants in a symbiotic relationship without causing any apparent diseases to the plants and they are one of the most promising sources of bioactive metabolites. Natural products from fungal endophytes have a broad spectrum of biologically active secondary metabolites and the recent literature is stating that 51% of the drugs isolated from endophytic fungi were previously unknown.

In our project, altogether 707 fungi have been isolated from eight different medicinal plant species in Hungary. The endophytic fungi from the parts of sampled plant were isolated and purified as well as started to identify. Then the individual strains were cultivated in a liquid medium and extracted sequentially with organic solvents for both the targeted and non-targeted screening the bioactive secondary metabolites. During the targeted screening, the endophytic fungi *Epicoccum nigrum* and *Alternaria sp*., isolated from the plant *Hypericum perforatum* has been identified as the producers of the host plant metabolites, hypericin and emodin. The presence of these compounds was qualitative and quantitative analysed using HPLC-UV analysis, which was further confirmed by UHPLC-HRMS and UHPLC-HRMS/MS techniques by comparing them with authentic standards and database entries. In the case of the non-targeted screening, the crude extracts of endophytic fungi isolated from various sources were screened for their antimicrobial activities against different bacterial pathogens for the purposes of the future purification of the active metabolites.

Our results could reveal that the endophytic fungi are one of the potential sources for the compounds, which have remarkable importance for pharmacological application.

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Incompatible symbiotic interactions between *Sinorhizobium meliloti* strain Rm41 and the ecotypes of the host *Medicago truncatula*

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Legumes develop a symbiosis with nitrogen-fixing soil bacteria called rhizobia. In this relationship, legumes provide an optimal environment and energy source for rhizobia. In return, rhizobia fix atmospheric nitrogen into ammonia for the plants.

The legume-rhizobial interaction begins with a molecular dialogue between the two partners. Flavonoid are released into rhizosphere by legume roots, attract rhizobia and induce the synthesis and secretion of Nod factors. Nod factors induce the elongation and curling of the root hairs which trap rhizobial bacteria. Within these trap sites, infection threads are initiated and extended, through which the bacteria are released into new cells formed by the division of cells in the nodule primordium the meristem originated from cortical cells. In *Medicago truncatula*, the meristematic cells keep dividing and the infected cells and the bacteria therein develop into symbiotic cells forming the root nodule.

The nitrogen-fixing symbiotic relationship is highly selective: Particular rhizobial species or strains establish an efficient symbiosis with only a limited set of legume species or genotypes. In nature, *S. meliloti* developes a symbiosis with *Medicago* species like *M. truncatula* and forms root nodules. Interestingly, certain combinations of *S. meliloti* strains and *M. truncatula* ecotypes that otherwise form effective symbiosis with other partners lead to symbiotic incompatibility, i.e. nodule development is arrested.

We have screened more than 100 ecotypes of *M. truncatula* with different *S. meliloti* strains and identified incompatibility between *S. meliloti* strain Rm41 and *M. truncatula* ecotypes F83005 and Jemalong. To find out the reason, we performed random transposon mutagenesis of Rm41 and overexpressed all predicted ORFs of strain Sm1021 in Rm41. In this way, we identified a mutation that restored compatibility with F83005 and isolated a gene that could rescue normal nodule development in Jemalong. At present, we characterize both genes to find out how they contribute to the success of the symbiotic intercation.

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Characterizing the activity of high fidelity Cas9 nucleases and polymerase III promoters widely used for expressing gRNAs to improve gRNA design

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Cas9 nucleases are components of the CRISPR system and by making a complex with a small RNA (guide RNA) they can specifically cleave the target DNA, and by this enable editing of complex genomes. The specificity of nuclease is defined by the 5 ,end of the RNA molecule by its complementarity to the DNA sequence and the specificity can be easily reprogrammed by modifying this sequence. This simplicity is why they could make widespread and revolutionary changes in the field of molecular biology and genetic engineering over the past few years.

Last year we found that extending the guide RNA with an extra 5' G nucleotide diminishes the activity of the increased fidelity Cas9 variants (Kulcsár et al. 2017). For making use of the wild type SpCas9, this modification is routinely applied to guide RNAs starting with a non-G nucleotide in order to be effectively transcribed from the U6 promoter.

The U6 promoter is widely used for the expression of small RNAs such as Cas9 guide RNAs. The transcriptional initiation of the human U6 promoter is not precisely defined. It is generally accepted that RNAs are efficiently transcribed only from a starting guanine nucleotide, so that the length of the transcribed RNA molecule is influenced by the occurrence of the first guanine. It is an important issue when exploiting the increased fidelity SpCas9 nucleases to modulate cellular functions, as we just showed in our recent study.

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However, the precise nucleotide preference for the transcription initiation of the U6 promoter is not fully understood. Beside getting a more comprehensive picture of the U6 promoter, we aim to learn the preferences of another promoters. To study more systematically the sequence preferences of these promoters for initiating efficient transcription, I created RNA libraries and using next generation sequencing I will determine the sequence specificity of the promoters, thereby enabling more effective guide RNA sequences to be designed.

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Molecular examination of smoking-related endothelial dysfunction in umbilical cord vessels

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One of the most typical exogenous stress factors during pregnancy is the smoking. As cigarette smoke is known to contain a large number of prooxidants (*e.g.,* free radicals, heavy metals), many of the adverse effects of smoking may result from oxidative damage to critical biological substances. The effects of smoking on the adult body have been well studied, but the molecular background of the effects on the fetal development is poorly understood. A better understanding of the pathophysiological complications emerging during pregnancy necessitates detailed studies of the umbilical cord (UC).

Therefore, the object of our work was the UC, which is primarily responsible for the transport of oxygen and nourishment to the fetus. The UC vessels can be considered as direct elongation of the fetal vascular system, and particularly exposed to harmful agents that are not filtered out by the placenta. Our aim was to find adequate procedures to detect the impact of maternal smoking on intrauterine life. Our experiments were built around the endothelial nitric oxide synthase (NOS3). Because the UC vessels lack innervation, the endothelial cells (ECs) derived nitric oxide have crucial role in controlling blood flow and maintaining physiologic conditions. UC samples were used for morphological and immunohistochemical analysis. Structurally we found abnormal morphology and unequivocal devastated conditions of the ECs derived from neonates born to smoking mother (Sm). We found that the NOS3 activity was significantly dropped in Sm samples. Furthermore, with classic biochemical approaches on isolated vessels, we found elevated levels of prooxidants such as peroxynitrite, hydrogen peroxide and superoxide radical and, in parallel, inadequate response in antioxidant enzyme defence system's activity. Our results are indicating that the structural changes within the EC layer are closely related to the loss of essential regulatory functions. This effect is markedly driven by the increased oxidative stress and macromolecular damage, as a vicious circle. The long-term harmful exposure and the resulting lack of stress response can ultimately lead to endothelial dysfunction.

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