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DISSERTATION SUMMARIES

Biogas production from chicken manure

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Biomass based technologies have several benefits: the necessary substrates are available in large quantities, the generated energy carriers are environmentally friendly, easy to carry and store. Biogas is a renewable energy carrier which is similar to natural gas and can be used in practically all applications to replace natural gas. It is produced by anaerobic fermentation of the organic materials. The problem of poultry waste management has been an increasing concern in many parts of the world due to the huge amount of this waste stream. Pollutants from improperly managed poultry waste can cause serious environmental problems in terms of water, air, and health quality.

Chicken manure (CM) contains two main forms of nitrogen: uric acid and undigested proteins, which represent 70% and 30% of the total nitrogen in CM, respectively. Anaerobic decomposition of uric acid and undigested proteins in CM results in high amounts of unionized ammonia and ammonium ions. Excess ammonia inhibits the anaerobic microbial community and thus methane production. Because of the regular antibiotic treatment in the large-scale chicken plants, first we compared two types of CM containing straw bedding: samples were taken from broiler chickens receiving antibiotic treatment and from untreated chickens. The wide-spectrum antibiotics employed at the chicken farms negatively affected the biogas producing microbial community leading to rapid process failure. The antibiotic-free substrate was more suitable for microbial decomposition. Increasing the organic loading in the batch reactors led to elevated biomethane yields. Without antibiotics the system was more stable and it produced significant amounts of biogas for a longer period. The antibiotic-free substrate was used in the subsequent experiments.

The possibility of washing CM with water was tested next with the aim of decreasing the inhibitory nitrogen content of the substrate. After two days soaking CM in tap water, the liquid and solid phases were separated and significant nitrogen content was detected in the water phase. Removal of excess nitrogen-containing compounds improved the suitability of CM as sole substrate for anaerobic digestion. Biogas fermentation experiments were carried out with washed CM at mesophilic temperature (37 °C) in batch and continuous operational modes. The results demonstrated that anaerobic fermentation became sustainable when the reactors were fed with washed CM, which confirmed that ammonium concentration was indeed the limiting parameter in the anaerobic digestion of CM.

The simple and inexpensive method of removing nitrogen-rich water soluble components from CM, however, resulted in a large volume of water with high nitrogen and other dissolved organics content as a residual waste. In order to improve the economic value of the process, the separated liquid phase (CM-water) was used as a nutrient solution for algae because algae need a significant amount of nitrogen source for growth. A *Chlorella sp.* strain was cultivated in these experiments. The extreme dark color of the CM-water fraction did not permit its direct use for algae cultivation. In order to avoid light limitation CM-water was further diluted with water. At the optimal dilution ratio (CM-water : distilled water) the *Chlorella* culture grew vigorously and reached higher optical density than in its default growth medium. The harvested algal biomass could be recycled into the biogas generating process.

CM has low C/N ratio which is not favorable for evolving biogas with high methane content. To solve this problem we developed a co-fermentation scheme using the solid fraction of washed CM and corn stover, to increase this ratio. These experiments yielded promising results.

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Genes of the LATERAL ORGAN BOUNDARIES domain transcription factor family in *Brachypodium distachyon*

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The fascinating morphological plasticity of higher plants and formation of efficient shoot and root architecture for adaptation to continuously changing environment is ensured by their ability to establish new organs during their entire lifespan. This unique feature of plants is maintained by the reactivation of cell cycle in certain cells of differentiated tissues and by dynamic regulation of meristamatic cell activity as well (Birnbaum et al. 2008; Bhojwani et al. 2013).

The precise coordination of division and differentiation of indetermined cells is essential in respect of proper and proportional organogenesis. Researches on plant development showed that special cells at boundaries between parental meristems and developing primordia play a dual role in this process by separating and maintaining meristem and organ domains. In accordance with their special function these cells express unique set of genes that reduce cell division and auxin efflux carrier activity but activate meristematic gene expression at the same time (Rast et al. 2008).

LOB-domain (LATERAL ORGAN BOUNDARIES DOMAIN) genes are among typical genes expressed prominently by these boundary-forming cells. They encode a family of plant specific transcription factors characterized by the 100 amino acid long conserved LOB-domain structure being responsible for DNA-binding and protein-protein interactions (Matsumura et al. 2009). Although their exact function is mostly unknown, observations up to now suggest that LOB-domain proteins are involved in almost all aspects of plant development from germination to seed production (Majer and Hochholdinger 2011). Their importance on organogenesis has been studied so far mostly in *Arabidopsis thaliana*, notwithstanding that revealing their role in monocots might be at least so relevant and interesting both for scientific and agronomical considerations, too. Therefore, we aimed to get to know in detail the processes controlled by LOB-domain protein coding genes (LBDs) in *Brachypodium distachyon*, a recently accepted and widely used model plant for cereals with high agronomical importance (Draper et al. 2001).

In current *Brachypodium* genome database we identified 28 LOB-domain protein coding genes. The encoded proteins are clustered into two major classes and some minor subclasses can be distinguished on the basis of their amino acid sequence homology. At first we characterized the relative expression levels of *Brachypodium*- LBDs by quantitative-real-time PCR in dozens of plant parts from root tip to shoot apex, both in vegetative and generative organs. According to our uniquely detailed analysis, LBD genes show variety of tissue specific expression: some of them are definitely active in flowers, in developing seeds and different parts of the floret, some of them have high activity in green plant parts while some others can be described as root specific genes, supporting the extremely diverse function of LBD gene family. Moreover, we got several expression patterns which have a good correlation to the transcriptional activity of their homologues from other species (e.g. *Arabidopsis thaliana* or *Oryza sativa*) that strongly suggests evolutionary conserved function of LBDs.

Aside from few exceptions, the closely related genes clustering into same subgroup showed overlapping expression pattern suggesting potential functional redundancy among them. However, one of the most interesting examples for exceptions are the Bd2g3450 and Bd2g53690 genes which have significantly divergent transcript profile from each other despite of their very close phylogenetic relationship (expression of Bd2g34520 is restricted to root tips while activity of Bd2g53690 is especially high in generative organs). For further exploration of possible functions we have selected these two LBD genes, with special regard on their presumable direct connection with the cell cycle regulating machinery.

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Birds of a feather - comparative gene expression analysis of Ada3 - a meta-analytic review with experimental flavors

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In eukaryotes, alterations of the chromatin structure have an important role in silencing and activation of particular genomic regions. The epigenetic processes are involved in changing the genes expression patterns, therefore the cell is capable of responding to different environmental impacts. Posttranslational modifications of histone proteins are crucial in structural changes of the chromatin. One of the most frequent covalent histone modifications is the acetylation of lysine side chains. The GNAT (GCN5-related N- acetyltransferase) complexes (which contain GCN5 protein as a catalytic component) have a common subunit called ADA3 (alteration/deficiency in activation 3). The homologues of this protein can be found all over in eukaryotes, from yeast to humans.

Lots of experimental data bits can be found which show that ADA3 protein is a lot more than a simple adaptor subunit: although it doesn't have any catalytic activity, it may have a sophisticated role in the function of the multiprotein machineries it is involved in. ADA3

protein may have a direct contact with various nuclear receptors (e. g. T3, glucocorticoid receptors, retinoid receptors, ER (estrogen receptor)), with some signaling pathways (by the interaction of IL-1, and beta-catenin), also with other proteins such as p53 and p300. ADA3 protein was even found to be a target of HPV E6 oncoprotein. Presumably, ADA3 protein is a pivotal contributory partner in many gene expression regulatory processes and abnormal function and regulation of ADA3 may cause severe impairments, such as developmental disorders, or oncogenesis. Since our group's main area of interest is to gain insight into the relationship between histone modifications and tumors, ADA3 is a promising object of investigation.

Previously, the promoter sequence of the *Ada3* gene has been identified in *Drosophila melanogaster* (*dAda3*). On the basis of *in silico* analysis, some truncated promoter variants were produced. The functionality of these promoter fragments were comparatively examined in luciferase reporter gene expression assays, applied in S2 *Drosophila* embryonic cell culture. Using this approach, promoter segments were shown to affect the transcription of the reporter gene, both positively and negatively. Within these segments predicted binding sites were identified, which are dedicated to three well-known transcription factors, namely the *Hunchback*, *Hairy* and *Deformed*.

To describe the ontogenetic expression pattern of the *dAda3* in details, the mRNA level of this gene was measured by qPCR in 15 different developmental stages. The results show that the initial (maternal) amount of the Ada3 transcript is fairly decreased by the time of hatching. Both at the beginning and the end of the prepupa stage, definite increasing was observed, which both coincide with "ecdysone peaks" well-known from developmental biology of fruit fly.

Querying various databases, genes with spatiotemporal expression similar to *dAda3* were identified. According to high-throughput ChIP assays, within the upstream regions of these genes there are resembling TF binding patterns. Meta-analysis of public transcriptome datasets from NCBI GEO revealed numerous additional genes with *dAda3*-related expression. All these genes were used as a core of a predictive interaction network. Strikingly, beside physical and genetic relationships, the representation of GO terms clearly emphasize novel potential functions.

Our experimental results and the analysis of related bibliographic data suggest that *dAda3* has important roles in some fundamental mechanisms like cell cycle, gametogenesis, neuronal differentiation, oxidative stress response and ecdysone pathway. Although the exact mechanism of these interactions is not known, these predicted functions are expected to be conserved in a wide range of eukaryotic organisms. Thus, our subsequent research is planned to focus on a specific area of this subtle commitment.

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No evidence that bacterial gene regulation has adapted to mitigate the accumulation of toxic metabolites

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It has often been suggested that removal of toxic small molecules from the cell is necessary to the survival of the organism. In case of toxic intermediate metabolites, prevention of accumulation helps averting cellular damage. One potential strategy to achieve this is the tight regulation of producing and consuming enzymes around the damaging metabolite. In yeast, it has previously been suggested that toxic intermediate metabolites have their enzymatic genes clustered on the chromosome to promote such coregulation. However, a direct link between coregulation and metabolite toxicity remains unestablished. Here we focused on Escherichia coli to systematically test this theory by making use of a unique toxicity prediction algorithm that has been specifically designed for this organism. We tested two possible strategies to achieve tight coregulation for metabolic genes around toxic intermediates: co-regulation via shared regulators and allocation of genes within the same operon, a bacteria-specific mechanism of strong co-regulation. We show that while toxic intermediates have their neighbouring genes more often in the same operon than expected by chance, this effect is not general bur rather confined to few metabolic pathways. Furthermore, coregulation or mRNA-level coexpression of neighboring enzymes occur with similar frequency in case of both toxic and non-toxic intermediates. Taken together, even in an organism with huge population size, we failed to find any general signature of adaptation to specifically enhance co-regulation of genes participating in the production or consumption of toxic intermediate metabolites. These negative results could indicate that most potentially harmful metabolites do not reach toxic concentrations under physiological conditions. Alternatively, the lack of regulatory mechanisms to avoid toxic metabolite accumulation might be owing to suboptimal gene regulation, which appears to be widespread in bacteria.

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Evolution of robustness to protein mistranslation by accelerated protein turnover

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Translational errors occur at high rates, influence organism viability and the onset of genetic diseases. To investigate how organisms mitigate the deleterious effects of protein synthesis errors during evolution, a mutant yeast strain was engineered to translate a codon ambiguously (mistranslation). It thereby overloads the protein quality control pathways and disrupts cellular protein homeostasis. This strain was used to study the capacity of the yeast genome to compensate the deleterious effects of protein mistranslation. Laboratory evolutionary experiments revealed that fitness loss due to mistranslation can rapidly be mitigated. Genomic analysis demonstrated that adaptation was primarily mediated by large-scale chromosomal duplication and deletion events, suggesting that errors during protein synthesis promote the evolution of genome architecture. By altering the dosages of numerous, functionally related proteins simultaneously, these genetic changes introduced large phenotypic leaps that enabled rapid adaptation to mistranslation. Evolution increased the level of tolerance to mistranslation through acceleration of ubiquitin-proteasome mediated protein degradation and protein synthesis. As a consequence of rapid elimination of erroneous protein products, evolution reduced the extent of toxic protein aggregation in mistranslating cells. However, there was a strong evolutionary trade-off between adaptation to mistranslation and survival upon starvation: the evolved lines showed fitness defects and impaired capacity to degrade mature ribosomes upon nutrient limitation. Moreover, as a response to an enhanced energy demand of accelerated protein turnover, the evolved lines exhibited increased glucose uptake by selective duplication of hexose transporter genes. We conclude that adjustment of proteome homeostasis to mistranslation evolves rapidly, but this adaptation has several side-effects on cellular physiology. Our work also indicates that translational fidelity and the ubiquitin-proteasome system are functionally linked to each other and may, therefore, co-evolve in nature.

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Effect of essential oils and their main components on biofilm formation and quorum sensing of food-related microorganisms

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Food contamination and food poisoning are serious problems in the food industry and also in health care. Bacterial biofilms are a community of bacteria imbedded in a self-synthesized extracellular matrix. It can form easily on food surfaces and in processing environment, representing a problem of post-processing contamination and cross-contamination. These structures can be formed by bacteria and yeast as well, but the most common forms in nature are the mixed-species biofilms. Biofilm-forming bacterial cells are able to communicate by the density-dependent cell-to-cell communication mechanism called quorum sensing (QS). A variety of different molecules can be used as signals for communication. Common classes of signaling molecules are oligopeptides in Gram-positive bacteria, N-acyl homoserine lactones (AHLs) in Gram-negative bacteria and autoinducer-2 (AI-2) in both Gram-negative and Gram-positive bacteria. Potentially, the resistance of biofilm-forming microorganisms could be influenced by controlling QS and biofilm formation.

In the food industry conventional preservatives often produce unpleasant by-products. Therefore, it is important to find effective natural antimicrobial agents which replace the synthetic ones at least partially. Among others, various essential oils (EOs) could be used against food spoilage- and food-borne pathogens. EOs are volatile liquids obtained from herbs, spices and different plants mainly by steam distillation. Some of them have more than 50 different constituents. In some cases these oils can help prevent the formation of biofilms of both food-spoilage and food-borne pathogens and could be used as natural preservatives for the extension of shelf life of foods.

The present study focuses on the anti-biofilm forming and anti-QS effect of six EOs (cinnamon, clary sage, juniper, lemon, marjoram and thyme) and their main components (cinnamaldehyde, α-pinene, limonene, linalool, terpinene-4-ol and thymol) against five food spoilage bacteria, a food borne pathogen, a yeast and their mixed cultures. Besides lemon, EOs showed good anti-biofilm forming effect and also inhibited QS of *Chromobacterium violaceum* in most cases. Scanning electronmicroscopy images showed the disappearance of biofilm-specific structures, furthermore, the lab-on-a-chip measurements revealed quantitative changes in the protein profile of bacteria after these treatments. The biofilm formation of the food borne pathogen *Listeria monocytogenes* was also inhibited on chicken breast fillets after marinating it with thyme EO and thymol. Additionally, the oils added also had a pleasant flavour effect. In conclusion, the EOs tested are good candidates for food preservation and represent alternatives to synthetic additives.

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Extracellular lipase enzymes from zygomycetes fungi: production, isolation and examination of biotechnologically relevant properties

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Lipase enzymes (E.C. 3.1.1.3) hydrolyze the triacylglycerols, which are the major constituents of fats and oils, to produce free fatty acids, glycerol and partial acylglycerols. Moreover, many lipases can catalyze the synthesis and translocation of ester linkages resulting biotechnologically important ester compounds. Recently, there is a growing interest for microbial lipases due to their low production costs and wide range of industrial applicability. Accordingly, identification and biochemical characterization of novel microbial lipases have special importance for industrial process development purposes. Zygomycetes are good producers of lipase enzymes; however, only a few enzymes have been isolated and characterized from this fungal group to date. Our knowledge regarding to their synthetic activity in organic media is also limited.

In our studies, 204 zygomycetes fungi were tested on culturing media contained tributyrin for preliminary detection of their extracellular lipase activities. Many *Rhizomucor, Rhizopus, Mucor, Umbelopsis* and *Mortierella* strains showed high enzyme activity and selected for further submerged and solid-state fermentation assays. In those studies, effect of different inductor oils on the enzyme activity is also tested. Enzyme yield of some isolates was outstanding when wheat bran was used as supplement in both submerged and solid state fermentations. Addition of mineral salt solution and olive oil to the solid fermentation medium resulted in at least 1.5-fold increment in the enzyme activities. Lipase production was also tested using oat bran, pressed hempen-, line-, poppy-, pumpkin seed as substrate with high lipid-content. The pumpkin-, and poppy seed residues proved to be promising substrates for lipase induction.

Transesterification assays were performed in non-aqueous conditions using lyophilized crude lipases of selected 11 strains. Enzymes from *Rhizomucor miehei*, *Rhizopus oryzae, Rhizopus stolonifer, Mucor corticolus* and *Mortierella echinosphaera* exhibited the highest transesterification activity in *n*-heptane using *p*-nitrophenyl palmitate as fatty acid donor and ethanol as acceptor. To characterize the reaction, effect of incubation time and temperature, various reaction media and acceptor alcohol on synthetic activity were also tested. Results showed that prolonged incubation time and high temperature (40 and 50 °C) generally enhanced the product yield. Considering the results of fermentation and transesterification tests, purification and biochemical characterization of *R. miehei*, *Rh. oryzae*, *M. corticolus* and *Mo. echinosphaera* lipases were carried out. SDS-polyacrylamide gel electrophoresis indicated a molecular mass of about 52, 56, 20 and 30 kDa for the purified enzymes, respectively. Biochemical characterization assays including temperature and pH tolerance studies, substrate specificity determination, and examination of the effect of some ions, alcohols and organic solvents on the activity were also performed. Purification of lipase produced by *Rh. stolonifer* is in progress, and synthetic esters formed by transesterification and esterification reactions are also being researched using gas chromatography technique.

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Molecular characterization of ROP GTPase activated kinases in *Arabidopsis*

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The Rho-type GTPases have central roles in cellular processes associated with cytoskeletal dynamics (e.g. cell movement, cell division, cell shape, and cell polarity). These proteins operate as molecular switches: they activate signal transduction pathways when they are in GTP-bound conformation, but their signalling activity cease when they are GDP-bound. If the Rho GTPase is in the GTP-bound form, it can further activate a diverse set of downstream signalling effector proteins.

Plants has a specific group of Rho-type GTPases, the „Rho of plants" (ROP) family. Our knowledge about the signalling pathways associated with ROPs is yet incomplete. ROPs differ from other Rho-type GTPases in the regions which are responsible for effector binding, suggesting that ROP GTPases have specific effectors. Indeed, plants lack the Rho GTPase-activated PAK kinases, which are very important mediators of Rho GTPase signalling in yeast as well as in animals. Therefore our question was: are there any ROP GTPase-activated kinases, which may have PAK-like functions in plants? Due to a yeast two-hybrid screening approach two ROP-interacting kinases could be identified. These kinases interacted with the GTP- but not with the GDP-bound ROP form what is typical for ROP effectors. Furthermore, the in vitro activity of these kinases was dependent on the presence of GTP-bound ROP. These ROP-activated kinases belong to the subfamily VI of receptor-like cytoplasmic kinases (RLCKs) of *Arabidopsis*. They have a receptor kinase-like catalytic domain, but they don't have extracellular or transmembrane regions and that's why they can found in the cytoplasm. Based on their primary structure, the 14 *Arabidopsis* RLCK VI kinases can be classified into two groups (A and B). Only the members of group A have ROP GTPase-binding ability. Based on in silico comparison, several positions were identified where the amino acids are characteristically different in the sequences

of group A and B kinases. It was supposed that these residues might be responsible for the difference in ROP GTPase-binding. Although these amino acid motifs are dispersed along the kinase sequences, on 3D kinase models most of them form a common surface. We replaced these amino acids one by one in the ROP-activated RLCK VI_A2 kinase with typical amino acids of B-type kinases, using site-specific mutagenesis. Changing the motifs prevented ROP GTPase binding in yeast two hybrid system. The mutant kinases were produced in and purified from a bacterial protein expression system. The in vitro auto-phosphorylation activity of the kinase was completely eliminated in the case of certain mutations, while in other instances the activity become independent of the presence of the ROP GTPase. These results were confirmed with the RLCK VI_A3 kinase. As this kinase (in contrast to VI_A2) well phosphorylates the myelin basic protein substrate, we could demonstrate that the mutations affect substrate phosphorylation and auto-phosphorylation in the same way.

We were interested in how ROP-activated kinases are widespread in the plant kingdom. Therefore the evolutionary conservation of the position of ROP-binding amino acids was investigated in kinase sequences of different plant taxa with available whole genome sequence. We could conclude that RLCK VI A-type kinases exist in multicellular plants but not in unicellular algae. Consequently, their function is probably not essential for basic cell functions, but is rather required for the development of a multicellular organisms. This is supported by our experiments using transgenic and mutant plants with altered kinase expression. These studies show that the kinases regulate plant growth and morphology. That the in planta functions of these kinases are dependent on ROP GTPases, still need to be justified. The ROP-binding mutant kinase forms we produced may serve as a tool to answer this question.

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Cell response to UV irradiation: Bomapin is a new member in the damage response

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Ultraviolet light-induced DNA damage response is an extensively studied process because improper or delayed repair of the errors could lead to cancerous malformations. Although several proteins involved in DNA damage repair have been identified, the entire repair cascade and the protein-protein interactions remain to be explored. Based on the wavelengths UV light is classified as UV A, B and C. UV C irradiation is absorbed mostly by the ozone layer, therefore under normal circumstances, UV A and UV B are the major causes of DNA damage in epidermal cells. In order to study the process of DNA damage response upon UV B irradiation, we used keratinocyte cells to test their DNA repair abilities and analyze the possible mechanism which could lead to skin cancer progression.

In a primary screen we identified Bomapin as one of the most dramatically up-regulated gene in keratinocytes following UV B irradiation. Bomapin (SPB10) is a serine protease inhibitor which promotes cell proliferation under normal circumstances in hematopoietic and myeloid leukemia cells. In the absence of growth factors Bomapin can induce apoptosis. However, only a few pieces of data have been reported about the function of Bomapin. Based on these findings, it seemed to be interesting to determine whether this protein has a gain-of-function effect in cancer evolution. To learn about the possible biological function of *Bomapin* induction upon UV B treatment we used immortalized keratinocyte (Hker E6SFM, HaCat) and melanoma (A375) cell lines. As a first step we analyzed the kinetics of UVB induction by determining the mRNA level in different time intervals following irradiation (2h, 8h, and 24h). We found that *Bomapin* mRNA level was increased 2 and especially 8 hours after UVB irradiation (80 J/m^2), while 24h after the treatment the mRNA level was decreased in each the examined cell lines. The mRNA level of *Bomapin* was also increased upon a low intensity (20 J/m^2) UV B irradiation in primer keratinocyte cells. Accordingly, the transient change of mRNA level suggests that Bomapin might be involved in the UV B induced stress response. In accord with the elevated Bomapin mRNA level, higher amount of Bomapin protein could be detected after DNA damage induced by ultraviolet irradiation (2 h, 8 h, and 24 h) in Hker cells, suggesting Bomapin involvement in UV B damage response. Since the protein seems to be a possible player in UV B repair in the following step we investigated the subcellular localization of Bomapin protein. We found that under normal circumstances, Bomapin protein was mostly present in the cytoplasm and co-localized with tubulin. Few hours after DNA damage Bomapin is transported to the nucleus from the cytoplasm and it is detectable in chromatin bound foci suggesting its possible importance in the repair process of damaged DNA.

In summary, our results suggest that Bomapin protein could be an essential player in the UV B induced stress response. Further experiments are needed to clarify its regulatory function in UV B response.

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Detection of *Mycobacterium tuberculosis* complex ancient DNA in human skeletal remains

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The aim of this research was to detect the presence of the causative agent of tuberculosis (TB), the *Mycobacterium tuberculosis* complex (MTBC) in ancient Hungarian human remains, in order to obtain a better understanding of the prevalence and pathology of this devastating disease throughout different historical time periods.

We have analyzed skeletal remains dating from the Neolithic to the Late Medieval periods (including Bronze Age, Avar Age and Árpádian Age samples). All the skeletal series selected for investigation contained cases showing macromorphological skeletal alterations indicative of TB. Both classical/advanced stage bone lesions and atypical/early-stage symptoms were searched for. The bone material has been screened for the presence of MTBC aDNA both in morphologically positive and negative cases. The members of the MTBC contain a multicopy repetitive insertion sequence element called *IS6110*. The presence of TB aDNA was assessed by applying a PCR-based assay targeting the MTBC *IS6110* region. To increase the sensitivity of the assay, a nested PCR strategy was applied. Initially, conventional PCR was performed using primers *IS6110*F and *IS6110*R to generate a 123 base pair product. The examination was carried out in a special pre-PCR area with a stringent environment for the studies of aDNA including the use of protecting clothing, UV-light exposure of the equipment and bleach sterilization of the surfaces. The aDNA work has been carried out in the aDNA laboratory of the EURAC Institute for Mummies and the Iceman in Bolzano and the Archaeogenetics Laboratory of the Institute of Archaeology, Research Centre for the Humanities, Hungarian Academy of Sciences.

Our preliminary biomolecular results indicate high prevalence of TB infection in the Late Medieval population of Bácsalmás-Óalmás (Pósa et al. 2013). Further analyses were carried out in this particular series, however, has drawn attention to the complementarity of biomolecular and macromorphological investigations; in addition to double-positive cases, morphologically negative/PCR positive and morphologically positive/PCR negative cases have also came to light in the course of the examinations (Pósa et al. 2015a). As for the sampling strategies, remnants of MTBC aDNA were found in skeletons from both sexes and all age groups. Corresponding to the scientific literature, we have sampled compact bone for DNA extraction in each case. However, we have also made several successful attempts to follow protocols of sampling teeth for MTBC aDNA extraction (Faerman et al. 1999; Nguyen-Hieu et al. 2011; Pósa et al. 2012). We also carried out a paleomicrobial study of the Alsónyék Neolithic series. Relatively high prevalence of TB infection was proved in the grave group no. 13 (Pósa et al. 2015b). The PCR analyses of some of the included series are still in progress, similarly to the ongoing spoligotyping and sequencing of the previously extracted MTBC aDNA remains possibly providing more detailed information on the different MTBC pathogens.

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Unitary GABAergic volume transmission from individual interneurons to astrocytes in the cerebral cortex

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Communication between individual GABAergic cells and their target neurons is mediated by synapses and, in the case of neurogliaform cells by a unitary form of volume transmission. Effects of non-synaptic volume transmission might involve non-neuronal targets and astrocytes not receiving GABAergic synapses but expressing GABA receptors are suitable for evaluating this hypothesis. Testing several cortical interneuron types in slices of the rat cerebral cortex, we show selective unitary coupling from neurogliaform cells to astrocytes with a fast, $GABA_A$ receptor and GABA transporter mediated component and a slow component that results from the activation of $GABA_B$ receptors on neurons. Unitary GABAergic responses failed to produce Ca^{2+} influx in astrocytes but were able to significantly hyperpolarize the reversal of nearby $GABA_A$ receptor mediated synapses. Our experiments identify a presynaptic cell type specific, GABA mediated communication pathway from individual neurons to astrocytes suggesting a role for unitary volume transmission in the control of ionic and neurotransmitter homeostasis.

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Macroevolutionary investigations on fungal models: The origins of taxonomic diversification in the order Agaricales

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One of the hot topics of current evolutionary biology is macroevolution. Macroevolution basically constitutes the study of the mechanisms behind speciation, diversification and adaptation processes, which are thought to have shaped the biological diversity as we see today and its history presented by fossils. One of the central concepts is adaptive radiation, i.e. when explosive speciation takes place in a geologically short time. These processes have been extensively studied in plants and animals yet we have limited knowledge as to what were the key contributing factors to the functional, life-history, and ecological diversity displayed by fungi today. Therefore our research project targets one group of fungi, the order Agaricales based on previous results at our department to address these questions. The Agaricales is the most species-rich group of the Basidiomycota, numbering ca. 480 genera and 14,000 described species. However, the forces driving speciation and evolutionary diversification in these mushrooms are poorly known. Understanding why and how certain lineages became extremely species-rich, while others are only represented by a few species and determining the timing of major lineage expansions are questions of utmost importance in basic research with important implications in conservation and classification.

To address these questions, we use statistical models of lineage diversification in a phylogenetic framework. Modelling of diversification relies on a new two-gene dataset (referred to as diversity dataset) for ca. 3,000 species. We selected the nLSU and RPB2 loci, which are known to provide sufficient phylogenetic information for relationships at the infrageneric level. In addition to the diversity dataset, a phylogenomic dataset is also being produced from the accessible whole genomes of basidiomycetous fungi, meaning about 60 to 100 species. We will use these two datasets to examine general patterns of speciation and extinction, to identify shifts in diversification rates and whether transitions between different fruit body types have an effect on speciation and extinction rates. We compiled a taxon database for all accepted genera in the Agaricales, which we used to estimate the appropriate species number per genus for a balanced sampling strategy scaled to 3,000 species. Preliminary analyses highlighted the patchy distribution of LSU and RPB2 in GenBank, which makes balanced sampling and dataset-assembly for large-scale investigations difficult. To overcome this, during the ADiv we are generating multiple LSU and RPB2 sequences using standardized protocols, resulting in universally applicable sequences that can be later leveraged for other projects too, including barcoding. In the first two years, we isolated DNA from 2,200 species, and sequenced the first ca. 1.5 kb of the LSU gene in 1,400 of these. In the third year, we continue with sequencing and commence diversification analyses using our datasets.

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Characterizing the dynamics of stationary vegetation boundaries

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The vegetation of landscapes consists of patches and intervening boundaries. Boundaries are often considered as the hotspots of vegetation changes, since several plant species reach the limit of their local distribution at the boundaries. Therefore, the dynamics of boundaries can be a sensitive indicator of environmental changes and can help to understand the mechanism of landscape scale pattern changes.

The dynamics of boundaries is usually described by their positional movements. Positional boundary dynamics has three main types: stationary, directional and shifting. A boundary is stationary, i.e. stable in position, if strong and stable abiotic parameters maintain them over long periods of time. Directional dynamics means the unidirectional translocation of boundaries due to abiotic or biotic drivers, while shifting dynamics takes place if these factors fluctuate in time.

In my study, I aimed to refine this scheme by analysing the dynamics of two vegetation complexes. The first one was located in Bugacpuszta, a landscape dominated by sand dune ranges with xeric plant communities on dunes and mesic ones in dune slacks. The other location was the Turjánvidék, which is a mosaic of wet meadows and steppe fragments. In both cases, the emergence of boundaries is dependent on topography-driven differences in water supply; therefore stationary boundary dynamics is expected to prevail. However, in the first case the mesic patches gradually lost the majority of their water supply in the last few decades, meaning that a key environmental factor had a directional trend. In the Turjánvidék, the average water supply is still satisfactory, but its inter-annual variations have become much larger, which can be considered as a shifting effect.

In Bugac, the vegetation of a 55-m and a 30-m transect were monitored between 1999 and 2013 using contiguous grids of quadrats. Presence/absence data of all plant species was recorded and the data were analysed with the split moving window technique. The method identifies the position of boundaries and the contrast the boundaries bridge between the patches. Five significant boundaries could be detected along the transects over the years. I tested the dynamics of their position and contrast using linear regressions. According to the results, positions did not follow the retraction of the groundwater, but the contrast between the adjacent patches showed significant trends: two boundaries disappeared and one new one appeared, which could be explained by the increasing proportion of xeric species and by the opening up of the vegetation.

In the Turjánvidék, I surveyed the vegetation along thirteen permanent, 40 m long transects. The surveys were performed in a wet year (in 2013) and in a dry year (in 2014). After performing the split moving window analysis, the boundary parameters were compared between the two years using t-tests. The results indicated that the position of the boundaries did not react to the differences of water availability, but the wet and dry patches became more similar to each other in the dry year, which could be traced back to the higher proportion of xeric species in the wet patches in 2014.

Thus, it can be concluded that even strong directional or shifting environmental processes cannot elicit positional changes in stationary boundaries; therefore positions are not recommended for bioindication in these cases. On the other hand, contrast changes sensitively indicated the reactions of the vegetation. This also means that landscapes with stationary boundaries can also be dynamic, but the typical pattern changes are not patch expansion vs. shrinking but patch fusion and division, which should be taken into consideration in a variety of ecological and nature conservation applications.

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Genetic investigations in neurodegenerative disorders

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Genetic investigations are useful tools to identify genes, which directly or indirectly play a role in the pathomechanisms of diseases and thereby indicating useful therapeutic interventions. The aim of our research group was to investigate different single nucleotide polymorphisms (SNPs) in multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). As a first step a biobank in the Department of Neurology, Szeged was established. Necessary ethical and biobank licenses were obtained and sample collections were started. Parallel with the collection of blood samples, databases belonging to the samples (age, sex, disease onset, etc.) were created. DNA purification from whole blood was performed using a simple salting out technique developed by Miller. To distinguish the alleles of the examined genes fluorescent labeled Taqman probes or restriction fragment length polymorphism techniques were applied. For statistical evaluation of the data SPSS software version 20 was utilized.

In case of MS chemokine receptor V polymorphism rs333 was studied, since earlier results about its involvement in the pathomechanism of MS were controversial. We investigated the receptor and its deletion in a large MS (428) and control (831) population from Hungary and Serbia. There was no significant difference in genotype (OR=1.092, 95% CI=0.807-1.478, p=0.568 for wt/wt (wt=wild type allele) vs wt/Δ32, Δ32/Δ32 (Δ32=Δ32 base pair deletion allele)) or allele frequency (OR=0.914, 95% CI=0.692-1.207, p=0.525). Neither the deletion

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nor the wt allele affected the Expanded Disability Status Scale score or the age at onset. Our results indicate no association between the chemokine receptor V Δ32 allele and MS.

For the investigation of pathomechanism of PD two different genes were analysed, vitamin D receptor which encodes a transcription factor that influences calcium homeostasis and immunoregulation, and the kynurenine-3-monooxigenase, which is the key enzyme of the kynurenine pathway.

In the vitamin D receptor study 100 PD patients and 109 healthy controls from the Hungarian population were genotyped for four polymorphic sites (BsmI, ApaI, FokI and TaqI). Our results demonstrate an association between the FokI C allele and PD, since the frequency of the C allele was significantly higher in PD patients than in controls, suggesting that this polymorphism may have a role in the development of PD in Hungarian patients.

There is substantial evidence that the kynurenine pathway plays a role in the normal physiology of the brain and it is involved in the pathology of neurodegenerative disorders. 105 unrelated, clinically definitive PD patients and 131 healthy controls were enrolled to investigate the possible effects of the different alleles of kynurenine-3-monooxigenase. None of the four investigated SNPs proved to be associated with the disease or with the age at onset. The investigated SNPs presumably do not appear to influence the gene function and probably do not contain binding sites for regulatory proteins. This was the first study to assess the genetic background behind the biochemical alterations of the kynurenine pathway in PD, directing the attention to this previously unexamined field.

Evidences indicate that there are aberrations in vitamin D endocrine system in ALS too. Our aim was to investigate SNPs from vitamin D receptor gene in 75 sporadic ALS patients and in 97 healthy controls. One of the four investigated SNPs was associated with the disease, but none of the alleles of the four examined SNPs influenced the age at disease onset. The ApaI A allele was more frequent in the ALS group compared to the control group, so it may be a risk factor for getting the disease.

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Identification of novel genes involved in the virulence of *Candida parapsilosis* during the generation of deletion library

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Recently, the increase in the prevalence of fungal diseases has focused attention on understanding the interactions between the pathogens and the host. Despite the growth of sequence information, a large number of fungal genes are uncharacterized and the function of genes are based solely on sequence homology. To characterize gene function in fungi such as the opportunistic pathogen *Candida parapsilosis*, gene knockout methods can be applied. In our previous work we have identified several fungal genes using RNA-Seq data that were overexpressed during host-pathogen interactions. To investigate their functions the creation of a knock out library was prepared. We have adapted a gene knock out strategy from the work of Noble and Johnson (2005). Fusion PCR method was applied to generate gene specific deletion constructions in order to disrupt genes from the genome of *C. parapsilosis* CLIB leu-/his- auxotrophic strain. Primarily we generated the flanking PCR products for the upstream and downstream regions for each of the genes, and the HIS1 and LEU2 marker PCR products. We used HIS1 marker from plasmid vector pSN52, and LEU2 from plasmid vector pSN40. Transformation of *C. parapsilosis* cells was performed chemically, using polyethylene glycol. For each of the identifications we used colony PCR to confirm the total deletion of the genes. All of the mutants were barcoded using a 20 bp tag in order to be able to identify them during later *in vivo* infections. All of the null mutant strains were tested under different conditions such as growth abilities on certain temperatures and medias, survival in the presence of cell wall, osmotic and oxidative stressors, and also pseudohyphae formation. Resistance to antifungal drugs such as fluconazole and caspofungin was also examined. We found null mutants that showed differences in appearance such as increased pseudohyphae formation and resistance to cell wall stressors (CPAR2 200390), regressed growth on different temperatures (CPAR2 303700) and alkali-phobic phenotype (CPAR2 100540). Difference in the virulence of these null mutant strains was also found when using infection models. CPAR2 200390 null mutants were found to be killed less efficiently by murine macrophages *in vitro*. In contrast, null mutants of CPAR2 303700 were killed similarly, however phagocytosed less than wild type cells by macrophages. Furthermore, more murine macrophages were found to phagocytose CPAR2 100540 deletion mutants, however the killing efficiency was lower comparing to the reference strain. In the future using this method we will be able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.

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Effects of systemic administration of L-kynurenine sulfate on behavior and on c-Fos expression level in C57Bl/6 mice

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L-Kynurenine (L-KYN) is a central metabolite of tryptophan degradation through the kynurenine catabolic pathway (KP). In the kynurenine catabolic pathway several endogenous metabolite exert neuromodulatory properties, of which kynurenic acid (KYNA) has been in therapeutic focus for more than two decades. KYNA can act as a non-competitive antagonist on 7nACh receptor, can exert dual action on AMPA receptors, and it can also competitively antagonize the NMDA receptor. Concomitant decreases in the concentration of extracellular glutamate, acetylcholine, dopamine and GABA can be observed, during the elevated KYNA level. The systemic administration of L-KYN sulfate (L-KYNs) leads to a dose-dependent increase in several downstream KP metabolite level, though in short term the most prominent change occurs in the concentration of the KYNA. An elevated level of KYNA can exert multiple effects on the synaptic transmission, resulting in complex behavioral changes, such as hypoactivity or spatial working memory deficits. Furthermore, a shift in the brain concentration of KYNA has been described in several neurodegenerative disorders e. g. Parkinson's disease, Schizophrenia and Alzheimer's disease. The L-KYN itself has a direct role in neuroinflammatory processes. In several studies neuroprotection was achieved through kynurenergic manipulation. Our research group's previous findings proved that, administration of L-KYN, KYNA or its derivatives can be neuroprotective in cerebral ischemic events. The behavioral effects of acut or chronic administration of L-KYNs are partially described. However, most of these observations emerged from studies focused on rats. Description of the behavioral effects of kynurenergic manipulations in mice are virtually lacking so, it is fundamental to investigate a dose-dependent impacts of acut L-KYNs administration in intact mice.

For this reason our aim was to investigate whether the systemic administration of L-KYNs (25, 100, 300 mg/bwkg) would produce alterations in behavioral tasks connected to locomotion (open field), anxiety (open field) and memory formation (object recognition) in C57Bl/6 mice, compare to the control group (0.1M PB). Then, to evaluate the changes in neuronal activity after L-KYNs treatment, we estimated the c-Fos expression levels in the corresponding subcortical brain areas. For this, we used that dose, which was found to exert the most prominent alteration in the behavioral tasks (300 mg/bwkg).

The L-KYNs dose-dependently affected the general ambulatory activity and the moving velocity of the mice. The lower doses led to hypoactivity, decreased the moving speed and the moving time, whereas the high dose led to hyperactivity, increased the moving speed, while decreased the moving time. Thus, the administration of the high-dose (300 mg/bwkg) altered the moving pattern also. The treatment dose-dependently increased anxiety-like behaviors, as peripheral zone preference of the open field arena progressively emerged and the rearing activity was gradually attenuated. The object recognition performance was not affected by the lowest (25 mg/bwkg) dose, whereas the higher doses completely abolished the formation of object recognition memory. Significant decreases in the number of c-Fos-immunopositive-cells was found in the dorsal striatum and in the CA1 pyramidal cell layer of the hippocampus. These subcortical brain areas can be linked to the regulation of moving speed and memory formation processes, respectively.

We could conclude that a single exposure to L-KYNs leads to dose-dependent behavioral disturbances, which might be related to the altered basal c-Fos protein expression levels in C57Bl/6j mice. In the near future we are planning to characterize different conditional kynurenine mutant mouse lines. Our present results can form a good reference to evaluate the behavioral disturbances of a genetically modified mice.

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