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DISSERTATION SUMMARIES

Evaluation of the effect of unsaturated fatty acids and irradiation on U87 glioma cell line

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Glioma is an invasive, aggressive form of brain tumors, with high rate of recurrence and resistance to radio and chemotherapy. Patients usually survive less than two years after diagnosis. The common treatment consists of surgical resection, followed with radiotherapy and/or chemotherapy. There is a great necessity for development of new therapeutic methods. Strategies are under development or clinical phase.

UFAs (unsaturated fatty acids) are one of the adjuvants that are applied as therapeutic agents for the treatment or alleviation of the symptoms of several diseases, like diabetic retinopathy, insulin resistance, inflammatory Bowel disease, cardiovascular diseases and several types of cancer. Numerous *in vitro* and *in vivo* studies prove the benefic effect of application of PUFAs (polyunsaturated fatty acids) as agents (solely or combined with chemo- or radiotherapy) in glioma therapy. Application of γ-linolenic acid on human glioma in clinical phase inhibited recurrence.

In order to determine the ideal type and concentration of UFAs as adjuvants in radiotherapy, we performed *in vitro* evaluation on U87 MG glioma cell line. We evaluated the effect of the following UFAs: arachidonic acid (AA, 20:4n-6), docosahexaenoic acid (DHA, 22:6n-3), gamma-linolenic acid (GLA, 18:3n-6), eicosapentanoic acid (EPA, 20:5n-3) and oleic acid (OA, 18:1n-9). Cells were treated with each fatty acid solely and in combination with 5 or 10 Gy.

We performed biochemical (LDH and MTS) and biophysical (RT-CES) assay to evaluate the effect of these fatty acids. We found that AA, DHA and GLA was more effective in sensitizing U87 cells to radiotherapy, so further experiments were performed with these three compounds, namely morphological, gene and miRNA expression analysis.

Statistical analysis of more than forty parameters based on holographic images was performed. Cell number and confluence was significantly diminished when they were treated with AA or when they were exposed to 10 Gy combined with AA, DHA or GLA. Application of PUFAs as adjuvants to 10 Gy caused significant alteration in cell thickness and irregularity, which indicate that cells have rounded and detached from the surface.

The molecular pathways that influence and determine the course of glioma when it is treated with UFAs (solely or in combination with radiotherapy) are not entirely deciphered yet. Thus, we decided to investigate the effect of AA, DHA and GLA at gene and miRNA expression level. Based on the scientific literature we have chosen to investigate gene expression on U87 cells that were solely PUFA treated or irradiated or co-exposed to PUFA and 10 Gy. We noticed significant alteration for at least one of these parameters in expression of endoplasmatic reticulum stress related genes (Grp78, DDIT3); genes which respond to oxidative stress (HMOX1, AKR1C1, NQO1), oncogenes (p53, c-Myc); early response genes (Egr1, TNF- , FOSL1, c-Fos); Gadd45a - a validated target in cancer treatment - and Notch1, a potential therapeutic target in glioblastoma. Out of the oxidative stress responsive genes that responded significantly to co-exposure to PUFA and 10 Gy HMOX1 is a potential target in glioma treatment, and NQO1 is a priority one.

Due to their small size and stability the study of the effect of miRNA on glioma therapy is an intensively investigated field (Low et al., 2014). We investigated the effect of AA, DHA, GLA and/or 10 Gy on U87 cells for the following miRNAs: miR34a, miR96, miR146, miR181a, miR148a, miR148b and miR152. Significant effect was noticed in case of miR146 and miR181a.

Our gene expression studies indicate that GLA and irradiation alter the expression of the therapeutic target Notch1 significantly. When 10 Gy is combined with AA, but not with DHA or GLA, changed the expression of several genes in a significant manner (p53, c-Myc, TNF-α and c-Fos). Our results confirm that UFAs are potent agents which enhance the effectiveness of radiotherapy.

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Synaptic changes in depression disorders

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Major depressive disorder (MDD) is predicted to become the leading cause of disability worldwide by the year 2030, representing an enormous financial and social burden. Clinical management of MDD is quite limited due mostly to the fact that the neurobiology of depression and the mechanisms of antidepressant therapy are still largely unknown.

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Depression and stress are associated with the loss of hippocampal dendritic spines of principal cells, contributing to hippocampal dysfunction. Hippocampal neuroplasticity mechanisms have the potential to mediate rapid mood change. Because pyramidal cell spine synapse remodelling vitally influences hippocampal activity, we hypothesize that major depression are associated with loss of hippocampal spine synapses. Recently, we have confirmed the validity of the new "synaptogenic hypothesis" of depression by demonstrating an inverse correlation between the number of synapses in limbic brain areas and the severity of depressive symptoms, both in animal models and in human beings. It is hypothesized that loss of synapses in depression is, at least partly, caused by prolonged stress and the resultant glutamate excitotoxicity, which could be prevented by antagonizing glutamate release in response to stress. In addition to their anxiolytic, anticonvulsant, muscle-relaxant, and sedative/hypnotic effects, benzodiazepines, such as diazepam, strongly inhibit glutamate release at high, pharmacological doses.

Postpartum depression is a serious clinical problem that affects approximately 10-15% of postpartum women during the six-month period following childbirth. Symptoms of postpartum depression are similar to those of a major depressive episode, exerting a severe impact on family functioning and mother-infant relations in this critical period of life.

To test our theory that remodeling of hippocampal spine synapses also occurs in postpartum depression, we utilized a rat pseudopregnancy model. Ovariectomized CD(SD) rats were subcutaneously implanted with continuous release pellets, providing pregnancy levels of estradiol and progesterone. After 21 days, the hormones were withdrawn and the ensuing week was considered as the postpartum period. "Pregnant" and "postpartum" rats were tested in the learned helplessness paradigm and the number of their hippocampal spine synapses estimated using electron microscopic stereology. Inescapable stress caused a severe loss of spine synapses in "postpartum" animals, while there were no synaptic changes in "pregnant" females. In line with synaptic alterations, performance of "pregnant" rats was significantly better in the active escape test compared to "postpartum" animals.

We can conclude that maintaining pregnancy levels of estradiol and progesterone prevents the synaptic and behavioral effects of inescapable stress, suggesting that the sudden decrease in ovarian hormone levels after childbirth plays a major role in predisposing to postpartum depression.

Our result presents a series of experiments, investigating whether diazepam is able to prevent helplessness and to protect synapses in the learned helplessness (LH) model of depression. Diazepam, when administered intraperitoneally to ovariectomized female CD(SD) rats dose-dependently decreased depressive symptoms in LH and demonstrated synaptoprotective effects in electrophysiological and morphological measurements.

These findings further support the synaptogenic hypothesis of depression and suggest that synaptoprotective treatment is able to antagonize the negative effect of stress on mood, which may be useful in the clinical management of patients with recurrent and/or treatment-resistant depression.

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Occurrence and importance of Aspergilli in agricultural products and clinical sources

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Aspergillus species are filamentous fungi which are widespread on agricultural products in subtropical and tropical areas of the world. Aspergilli are able to produce a range of mycotoxins, which can be harmful to animals or humans, including aflatoxins, ochratoxins, fumonisins and patulin. *Aspergillus flavus* is also an important pathogen of various cultivated plants including maize, cotton and peanut, and cause serious yield losses throughout the world. Since aflatoxin production is favoured by moisture and high temperature, *A. flavus* is able to produce aflatoxins in warmer, tropical and subtropical climates. According to recent studies, climate change accompanied by global warming affects the occurrence of fungi and their mycotoxins in our foods and feeds. A shift has recently been observed in the occurrence of aflatoxin producers in Europe, with consequent aflatoxin contamination in agricultural commodities in several European countries not facing with this problem before (Italy, Serbia, Slovenia, Croatia, Romania, Ukraine). Although aflatoxin contamination of agricultural products is not treated as a serious threat to Hungarian agriculture due to climatic conditions, these observations led us to examine the mycobiota and mycotoxin content of different agricultural products (wheat, maize, chili pepper, nut, etc.) collected from different locations in Hungary and Vojvodina. The surface-sterilized products were placed on selective media, and the isolated fungal strains were identified using morphological and sequence-based methods.

Aspergillus strains are among the most common organisms causing fungal keratitis in tropical and subtropical areas. The main risk factor for the infection is trauma by vegetable matter during agricultural activities. Among *Aspergillus* species, mainly *A. flavus*, *A. terreus*, *A. fumigatus* and *A. niger* have been isolated from fungal keratitis cases. During our study, 52 *Aspergillus* strains isolated from keratitis cases in South India were examined. Based on morphological studies, all isolates were classified to the *A. flavus* species. For the molecular identification, part of the calmodulin gene was amplified and sequenced. As a result, 46 isolates were identified as *A. flavus*, while four as *A. tamarii,* one as *A. terreus* and one was found to belong to the *A. pseudotamarii* species. That was the first case that *A. pseudotamarii*

was identified from a human infection. Antifungal susceptibility tests of clinical isolates were carried out using disc diffusion and E-test methods. The detected antifungal susceptibility values were mostly within the value ranges determined previously for *A. flavus* isolates, although the *A. pseudotamarii* isolate proved to be more susceptible to amphotericin B than either *A. flavus* or *A. tamarii.* Aflatoxin producing abilities of the isolates were tested in YES culture media, and determined by HPLC analysis. Most of the examined *A. flavus* isolates carry the MAT1 mating-type gene.

Further investigations of the genetic variability of the *A. flavus* isolates by UP-PCR, microsatellite analysis and mating-type locus gene (MAT) analysis, and aflatoxin producing ability testing using an ELISA method are in progress.

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The role of glutathione transferases in the stress tolerance of different plant species

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Physiological processes involved in detoxification have important role in agriculture (and so in plant biology), because plants are exposed to disadvantageous environmental conditions. Abiotic stressors, e.g. xenobiotics, heavy metals presented in the soil, and drought are able to launch the production of toxic by-products of metabolic processes (such as lipid peroxides) and harmful amount of reactive oxygen species in stress-exposed plants, which can cause reduced growth and decreased yields.

Glutathione transferases (GSTs) are a divergent enzyme family with two major *in vivo* detoxification functions in plants: conjugating toxic compounds with a glutathione molecule, thereby making them less harmful and promoting their compartmentalisation to the vacuole, and the glutathione-dependent peroxidase activity, which plays a role in maintaining membrane integrity under stress conditions. To examine the role of GSTs in the abiotic stress tolerance of different plant species, we used two experimental set-up.

First, two inbred lines of the cereal model organism *Brachypodium distachyon,* Bd21 and Bd21-3 were grown hydroponically, and were exposed to osmotic stress treatment for modelling drought stress. We observed the effects of osmotic stress to growth parameters, water status, enzymatic responses, and gene expression pattern of the plants. As results, we concluded that root growth of the *Brachypodium* lines differed (Bd21 had increased root growth, while it was reduced in Bd21-3). The water homeostasis of the two line were similar: both showed isohydric strategy during our experiments. We observed higher guaiacol peroxidase and glutathione transferase activities in line Bd21, and all examined enzymes showed induced activities during the osmotic treatment. For quantitative real-time PCR, six GST genes were selected based on our previous studies on wheat cultivars, expression data published in literature, and promoter sequence analysis. In line Bd21 we observed the induction of a wider range of genes under the osmotic stress, which indicates the importance of the selected genes in the detoxification process, and also suggests (according to the other parameters) that line Bd21 may be more tolerant to the applied osmotic treatment. In addition, we may conclude that both lines are highly resistant, compared to cereals previously studied in our research, so using *Brachypodium* lines for experimental purposes may give important results for cereal breeding.

Our other experimental system was equipped to examine the detoxification processes of bred poplar clones. Poplars (*Populus spp.*) are widely cultivated plants for their rapid growth and high biomass, and are increasingly used in scientific research as model organism of trees, and for phytoremediation purposes. Stress adaptation processes against heavy metals and osmotic stress were examined on three outstanding biomass producer poplar lines. Cuttings were grown hydroponically, and treated by copper, zinc, and polyethylene-glycol. We described the water potential of plants, the malondialdehyde content of shoots and roots, enzyme activities (guaiacol peroxidase, glutathione peroxidase, and glutathione transferase activities), amount of reactive oxygen (total intracellular ROS, superoxid radical) and nitrogen species (nitrogen oxide, peroxynitrite). Furthermore, we quantified the induction of ten transcripts, which probable are fundamental parts of the poplars stress adaptation processes. Among these were four glutathione transferases, two ABC transporters, three metallothioneins, and a phytochelatine synthase. Our results shows, that all three poplar clones are efficient in stress adaptation, but this properties have different molecular backgrounds. *P. deltoides* clones B-229 and PE 19/66 showed slightly lower water potential during zinc and hyperosmotic treatment, and in all treatments, they have significantly lower glutathione transferase activities, than *P. x canadensis* clone M-1. By contrast, B-229 and PE 19/66 clones are more effective to induce the gene expression of various components of the detoxification process, such as the GSTs. Based on our research, *P. deltoides* clones may be well utilized for phytoremediation purposes on heavy metal contaminated sites with good water supply, but under osmotically inappropriate circumstances further research needed to understand acclimatization processes.

During our work, evidence was found for the important role of GSTs in the stress responses of *Brachypodium* and *Populus*.

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Ecophysiological characterisation of a biocontrol *Bacillus subtilis* strain

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As conventional chemical pesticides considerably increase the environmental load of agricultural areas, serious efforts are made to find and develop effective biocontrol agents with no ecotoxicological risks. Good extracellular enzyme and antibiotic producing microorganisms could be excellent antagonists of phytopathogenic fungi and bacteria. *Bacillus subtilis* is a Gram positive, aerobic, endospore-forming, soil bacterium, which is able to produce various antibiotics and a broad spectrum of extracellular enzymes. This bacterium may produce various non-ribosomal oligopeptides, such as iturin, surfactin and fengycin. These cyclic lipopeptides have both antifungal and antibacterial effects. Previously, elevated protease and -amylase secretion was reported by Kurosawa et al. (2006) from streptomycin resistant *B. subtilis*. This phenomenon appeared in connection with spontaneous mutations in the *rpsL* gene encoding the ribosomal protein S12. The aims of our study were *(1)* to make an ecophysiological characterization of the isolated *B. subtilis* strain and *(2)* to prove the effectiveness of the simple approach of Kurosawa (2006) for generating a series of biocontrol strains without the need for induced genetic modification of the original bacterium.

After isolating several bacteria from soil samples and rhizosphere of tomato, the isolates were identified based on the partial sequencing of the *gyrA* gene. Sequence of the whole genome of one strain (B23), which showed the best biocontrol abilities, was determined and compared with the that of the *B. subtilis* type strain (DSM-10). Antibiotic production of the two strains was also compared by TLC analysis. By sequence analysis, several single-nucleotide polymorphisms were found in various genes involved in the antibiotic production. These changes are suggested to be responsible for the enhanced antibiotic production of the newly isolated strain.

From the B23 isolate, spontaneous streptomycin resistant colonies were selected. Chymotrypsin-type protease activity in the ferment broths of the streptomycin resistant strains were determined and compared with the B23 strain. From the 20 tested mutants the K2 strain was outstanding with its fourfold chymotrypsin producing activity. Among the spontaneous streptomycin resistant mutants, six showed significantly enhanced tyrosine-containing antibiotic production. *In vitro* antagonism of the B23 strain and its streptomycin resistant mutants against phytopathogenic microorganisms and some mycotoxin producing fungi were characterized. Elevated inhibition zones were detected in case of some important pathogens. Effect of metal ions (*i.e.* cadmium, copper, manganese, nickel and iron) and pesticides (*i.e.* 2,4-dichlorophenoxyacetic acid, carbendazim, chlortoluron and linuron) to the enzyme production and activity were also examined. Manganese had positive effect on the enzyme production, while the presence of pesticides had no inhibitory effect. Analysis of the antibiotic profiles in the presence of metal ions and pesticides produced very similar results. Effect of the carbon and nitrogen sources on the production of antibiotics was tested. Saccharose, glycerol, cellobiose, starch, Na-nitrate and proline elevated the production rate of the tyrosine containing antibiotics.

Kurosawa K, Hosaka T, Tamehiro N et al. (2006) Appl Environ Microbiol 72:71-77.

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Investigation of redox homeostasis and elements of abiotic stress responses in *Arabidopsis* model plant

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The growth and yield of plants are highly dependent on environmental factors. The extremes of these conditions act as stressors leading to the formation of reactive compounds in the cells and to the imbalance of redox homeostasis. Adequate stress responses may restore the redox balance. Growing evidence suggests a model for redox homeostasis in which the reactive oxygen species (ROS)-antioxidant interaction acts as a metabolic interface for signals derived from metabolism and from the environment during stress.

The aim of my research was to explore the elements of defence mechanism, with focus on the redox re-establishment of redox homeostasis. In this work, the effect of salicylic acid (SA) and salt stress were investigated using *Arabidopsis thaliana* L. Columbia ecotype (wild type), glutathione reductase (*gr*) and dehydroascorbate reductase (*dhar*) mutant lines which grown in hydroponics. In order to increase salinity tolerance, as a priming effect, plants were pretreated with 10^{-9}-10^{-4} M SA followed by 100 mM NaCl in long-term experiments.

The stress induces serious metabolic perturbations in plants, as it generates ROS which disturb the cellular redox system. In this study we examined the viability of cells and ROS level and its derivatives by fluorescent dyes. We determined the levels of antioxidants and the activities of some antioxidant enzyme such as total ascorbate (Asc) and reduced (GSH) and oxidized (GSSG) glutathione, glutathion reductase (GR) and dehydroascorbate reductase (DHAR), which are protecting plants against ROS damages. The amounts of Asc and GSH increased under stress conditions mainly at 10^{-7}-10^{-5} M SA concentrations also at mutants lines. In addition, maintaining a high ratio of GSH/GSSG showed to play an important role in SA and salt tolerance of *Arabidopsis* wild type and mutants. The activities of GR and

DHAR enzymes also contributed and helped to maintain the cell balance under stress conditions.

Most importantly, antioxidants provide essential information on cellular redox state and they influence gene expression associated with abiotic stress responses to maximize defense. We analyzed also the expression levels of GR and DHAR genes by real-time-PCR with focus on the role of GR and DHAR izoenzymes and our data also showed changes in their transcript levels under stress conditions and in the acclimatization process.

Redox reactions are the fundamental metabolic processes through which cells convert and distribute the energy that necessary for growth and maintenance. *Arabidopsis* plants transformed with a redox-sensitive GFP (roGFP) targeted to the cytosol (c-roGFP1) were used for monitoring the real-time redox status of the cytosol in SA and salt stressed plants. Utilization a flourometer to detect redox-related changes of roGFP has been demonstrated. The utilization of a fluorimeter enables the processing of many samples and it averages the whole tissue rather than only few cells within a tissue, as in the case of confocal imaging.

It is concluded that constitutively high level of reduced GSH are advantageous to act as a strong buffer against ROS but would make the system less responsive to changes in redox potential that may be needed to upregulate the inducible defence components. In this study we have adapted fluorometer reading and compared this assay with confocal imaging. Nevertheless, the data showed that roGFP is redox sensitive in plant cells and that sensor makes it possible to monitor, in real time, dynamic changes in redox homeostasis *in vivo*. During long-term experiments, we were able to apply this technology in combination with many aspects of the antioxidant defence system measurements to the analysis of redox changes in response to stresses or to various mutants.

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Protecting roles of 27 kDa heat shock protein Hsp27

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Hsp27 belongs to the small heat shock protein family, which are ATP-independent chaperones. The most important function of Hsp27 is based on its ability to bind non-native proteins and inhibit the aggregation of incorrectly folded proteins maintaining them in a refolding-competent state. Additionally, it has anti-apoptotic and antioxidant activities.

Several studies have shown cytoprotective effects of Hsp27 against reactive oxygen species. Doxorubicin is a widely used chemotherapeutic agent against several types of cancer. Beside it's cytostatic properties, doxorubicin has a severe cardiotoxic side effect. To study the cardioprotective effect of Hsp27 *in vivo*, a transgenic FVB mouse strain overexpressing the human Hsp27 protein was established. Transgenic mice and their wild type littermates were injected with a single dose of doxorubicin, control animals were treated with saline. We detected significant level of apoptosis in cardiac tissues of doxorubicin treated wild-type mice using caspase-3 immunhistochemistry and TUNEL (terminal deoxynucleotidyl dUTP nick end labelling) assay. However, the number of apoptotic cells were substantially reduced in Hsp27 overexpressing transgenic hearts. Caspase-3 western blot analysis also confirmed the cardioprotective effect of Hsp27 against doxorubicin. Using qPCR analysis, we found significant increase in the expression of proteasomal genes in wild-type hearts after doxorubicin treatment. mRNAs of proteasome subunit 3, Psmc3 interacting subunit and ubiquitin conjugase 4 showed the most remarkable increases. However, overexpression of Hsp27 did not repressed the expression of these genes, suggesting that cytoprotective effect of Hsp27 is not directly linked to proteasome function.

Hsp27 has well known neuroprotective effect as well. Previously, using APPxPSe1xHsp27 triple transgenic mice we have shown that overexpression of Hsp27 protein ameliorates certain symptoms of Alzheimer's disease. Alzheimer's disease (AD) model mice overexpressing Hsp27 showed reduced number of amyloid plaques and improved presynaptic and cognitive functions. In order to clarify the molecular role of Hsp27 in amyloid plaque number reduction, we monitored the gene expression of several genes potentially involved in ß-amyloid metabolism such as APP, ApoA1, ApoD, ApoE, LDLr, Lrp1, Lrp2, Hsp90, and neurodegeneration (NOS1 and NOS2) in the cortex of Hsp27 transgenic mice using qPCR. Expression levels of ApoD and Lrp2 were slightly increased (128% and 128%, respectively), in the brain of Hsp27 transgenic mice compared to wild type controls (100%), whereas there was no change in the mRNA level of APP, ApoE, LDLr, Lrp1, Hsp90, NOS1, and NOS3. Rather surprisingly, cortical expression of ApoA1 was reduced by half in Hsp27 transgenics versus wild type mice. Decreased ApoA1 expression in Hsp27 transgenic mice was further confirmed using western blotting. ApoA1 protein level was reduced in Hsp27 transgenic mice (61.1%), but slightly elevated in AD model mice (126.7%) compared to wild types (100%). However, AD mice overexpressing human Hsp27 protein possessed similar ApoA1 protein level than wild type mice, indicating that Hsp27 influenced ApoA1 expression .

A less studied aspect of Hsp27 mediated cell protection is its possible role in DNA repair mechanisms. Heat shock protein 27 have been reported to be overexpressed in various cancers and to associated with poor prognosis for survival in patients with cancer. Association of Hsp27 with UV light- and radiosensitivity in cancer cells was also shown by several studies. Phosphorylated Hsp27 can stimulate pentose phosphate pathway (PPP) via binding and activating glucose-6-phosphate dehydrogenase (G6PD). PPP is responsible for producing nucleotide precursors for DNA repair, and G6PD-deficient cells are impaired for DNA double strand break (DSB) repair. To study the possible

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role of Hsp27 in DSB repair mechanisms, qPCR analysis of non-homologous end-joining (NHEJ) and homologous recombination (HR) associated genes was performed. Total RNA was isolated and reverse transcribed from Hsp27 overexpressing B16 mouse melanoma cells as well as wild type B16 cells, then primer pairs for 32 different genes were used in qPCR analysis. We detected increased expression of breast cancer protein 2 (BRCA2) (222%), replication protein A3 (RPA3) (241%) and aprataxin (APTX) (192%) in Hsp27 overexpressing B16 cells compared with wild type B16 cells. Further analyses of protein expression of these genes are necessary in Hsp27 overexpressed and silenced B16 cells, in order to understand better the multiple role of Hsp27 in cancer.

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Regulation of protective proline synthesis during reactive carbonyl stress

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Enviromental stresses impact on all aspects of plant architecture and represent a serious challenge for developing sustainable agriculture at a time of significant growth in the global population. To cope with these stresses, plants have evolved a wide spectrum of molecular programs to sense change rapidly and adapt accordingly. Understanding, and - if it is possible – improving these reprogramming events under constantly changing environmental conditions has been a subject of great interest.

Plants have evolved diverse strategies of acclimatization and avoidance to cope with adverse environmental conditions. Proline, as free amino acid is common among stress-induced metabolites and has been shown to accumulate during different environmental stresses including drought, salinity, and oxidative stress; moreover proline level responses to certain biotic stresses. Several protective functions were attributed to proline, such as scavenging ROS, acting as osmoprotectant and maintenance of redox equilibrium. Due to its action as singlet-oxygen quencher and scavenger of OH• radicals, proline is able to stabilize proteins, DNA and membrans. The in vitro use of reactive carbonyls, like methylglyoxal or glycolaldehyde is a straightforward method to imitate the ROS mediated in vivo damages. To comfirm this theory, we examined the protective effects of proline on glycolaldehyde treated lactate-dehydrogenase. In these experiments, we used protein oxidation assay and *in vitro* activity measurements. We can conclude that proline can not directly protect this enzyme from oxidation in *in vitro* assays. Several *in vitro* enzyme activity measurements showed, that proline can protect that enzyme activity and may be it interact directly with the reactive carbonyl. The *in vivo* experiments were carried on *Arabidopsis thaliana* (Columbia ecotype). In *A. th*aliana the synthesis of prolin is perfomed by two enzymes, the P5CS2 acts as a housekeeping enzyme and the P5CS1 is the stress-induced one which is int he centre of our interest. Earlier in silico analyses showed that in the *P5CS1* promoter, transcription factor binding sites from G-Box and MYB families can be found. The yeast one-hybrid system is a powerful method to identify heterologous transcription factors that can interact with a specific regulatory DNA sequence of interest. In the course of the experiments on this gene we focused on its methylation pattern too, because these posttranscriptional modifications can cause significant alterations in gene expression. In the promoter fragment of P5CS1 next to the potential transcriptional factor binding sites, a theoratical small RNA binding site and a potential methylation site were identified. By the McrBc digestion of isolated plant DNA followed by PCR, we can make the methylation profile of the promoter and the gene body. Therethrough we can conclude that the abovementioned DNA fragment is the mostly methylated region of the promoter, may be it has an important role in the regulation of gene expression. We can alternate the methylation pattern by treating the plants with 5-azacitidine in vivo. This way we can have a more focused point on the relation between the methylation set(status) of the gene and its expression level. These results suggests that the methylation pattern of *A. thaliana* P5CS1 shows a dinamic phenomenon upon development and stress response.

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Sulfide oxidizing enzymes in a purple sulfur photosynthetic bacterium

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Phototrophic purple sulfur bacteria can utilize various reduced inorganic sulfur compounds (e.g. sulfide) as electron donor during anoxygenic chemoautotrophic photosynthetic growth. In these bacteria, flavocytochrome c and sulfide quinone oxidoreductase proteins oxidize sulfide to sulfur and supply the electrons into the photosynthetic electron transport chain. These ancient enzymes belong to the disulfide oxidoreductase protein family. Flavocytochrome c (Fcc) is a periplasmic enzyme consisting of a large sulfide-binding flavoprotein (FccB)

and a smaller, heme c binding cytochrome c subunit (FccA). Sulfide quinone oxidoreductases are monomeric membrane-bound flavoproteins which present in all domains of life. Sqr can transfer electrons from sulfide directly into the membrane quinone pool while Fcc reduces periplasmic c-type cytochrome proteins.

Thiocapsa roseopersicina is a photosynthetic purple sulfur bacterium. Three genes encoding sulfide oxidizing disulfide oxidoreductases were identified in the genome sequence: *fcc*, *sqr* and *sqn*. The Sqr and Sqn belong to group IV and group VI of the Sqr-type proteins, respectively. A detailed comparative biochemical, structural and functional analysis of these proteins is in the focus of this study.

The FccAB complex, the FccB, the Sqr and the Sqn proteins fused to Strep II affinity tag were expressed in *T. roseopersicina* strains. The recombinant flavocytochrome c variants and the Sqn enzyme could be purified to homogenity by affinity chromatography. In the absorption spectra of the oxidized and reduced forms of FccB, FccAB and Sqn, characteristic peaks of redox active flavin prosthetic group were identified. The flavin moiety apparently bound covalently to the proteins. The flavocytochrome c had also a redox active heme cofactor non-covalently bound to the FccA subunit. The Fcc variants were subjected to ultrafast fluorescence kinetic measurements in order to determine the interaction between the FAD cofactor and the protein. The affinity purified recombinant FccAB could oxidize sulfide and was able to reduce bovine heart cytochrome c at low sulfide concentrations. The temperature and pH dependences of the activity of the recombinant Fcc complex were determined: the optimal temperature was 45 °C while the optimal pH was 8.0. The FccAB was a moderately thermostable enzyme which had remarkable activity up to 60 °C. The recombinant Sqn and Sqr catalyzed the sulfur-dependent quinone reduction. The temperature and pH optima of quinone reductase activity of the Sqn were the same as determined for FccAB. Kinetic analysis of the Sqn activity at various pH revealed a lag phase preceding the reaction at high pH. This might mean that the enzyme needed activation for being able to reduce quinones at alkaline conditions. Additionally, the macromolecule structure of the Sqn was analyzed to explore the connections between the quaternary structure and the catalytic properties of the protein. Enzyme kinetic parameters of the Sqn disclosed that the enzyme affinity for sulfide was low as compared to other well-known sulfide quinone oxidoreductases. Consequently, Sqn might play role in the sulfide oxidation at high sulfide concentration. In contrast, the FccAB could have important function at low sulfide concentration in the sulfur metabolism in *T. roseopersicina*. The structural and functional analyses of the wild and mutant flavocytochrome c might lead to better understanding of the structure/function relationships of the disulfide oxidoreductase protein family. On the other hand, the biochemical and biophysical characterization of the Sqn should disclose specific properties of the group VI. of the Sqr-type proteins.

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Heavy metal induced nitro-oxidative stress in *Brassica* species

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Copper (Cu) and zinc (Zn) are essential micronutrients, which can be present in soils naturally or can be accumulated in the environment due to anthropogenic activities. Cu is a redox-active element, directly inducing the formation of reactive oxygen species (ROS) leading to oxidative stress. Zn, on the other hand, is a non-redox-active element, causing oxidative stress indirectly by the modulation of antioxidant capacity. Moreover, in excess, both metal trigger changes in the metabolism of reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite ($ONOO^-$) leading to nitrosative stress. The oxidative and nitrosative signalling interact with each other resulting nitro-oxidative stress during which the cellular functions damage by lipid peroxidation and nitration, protein carbonylation, tyrosine nitration and S-nitrosylation.

The primary goal of my study was to determine the degree of nitro-oxidative stress in two metal tolerant *Brassica* species exposed to Cu or Zn. Furthermore, I wanted to draw conclusions about the Cu- and Zn tolerance and phytoremediation usability of the species.

Nine-days-old hydroponically grown *Brassica juncea* and *Brassica napus* were treated with 0 (control), 10, 25 and 50 μM $CuSO_4$ or 0 (control), 50, 150 and 300 μM $ZnSO_4$ in nutrient solution for 7 or 14 days. Changes in microelement contents, formation of different ROS and RNS, cell viability, lipid peroxidation, cell wall alterations and enzymatic- and non-enzymatic antioxidants were examined in the root system.

Most of the Cu and Zn taken up by the plants were retained in the roots; however, the increment of Cu and Zn content within the *Brassica* shoots indicated an efficient translocation. Both metals in excess markedly modified the microelement homeostasis of *Brassica* plants. Both Cu and Zn treatment caused significant morphological alterations in the root system of *Brassica* species, e.g Cu and Zn were able to increase the lateral root number, especially in *B. juncea*, which may be part of a morphological adaptation process. A Cu concentration-dependent decrease of cell viability was also found after both 7 and 14 days of treatment; however in short term *B. juncea* root meristem did not show Zn-induced viability loss. Also, cell wall alterations were notable, since intensified lignification and callose formation were detected in the root system of Cu-stressed plants; however excess Zn caused only increased callose deposition.

Exposure to Cu induced nitric oxide generation in the root tips and this event proved to be dependent on the duration of the exposure and on the plant species. In short-and long-term treatments, *B. juncea* showed more significant activation of superoxide dismutase (SOD), inhibition of ascorbate peroxidase (APX) and oxidation of ascorbate (AsA) than *B. napus*. Moreover, hydrogen peroxide (H_2O_2)-dependent lignification was also observed in the Cu-exposed plants. In longer term, significant AsA accumulation and callose deposition were observed,

reflecting serious oxidative stress in *B. juncea*.

Due to the short-term Zn stress, SOD and APX showed higher activities in the roots of *B. juncea* keeping the amount of superoxide anion ($O_2^{\cdot-}$) and H_2O_2 at a control-like or lower level. Contrary, NO and $ONOO^-$ showed significant accumulation as the effect of Zn exposure. Despite the elevation of $ONOO^-$ levels, there was no detectable lipid peroxidation, which may indicate that it has a role in stress tolerance in *B. juncea* roots.

In the background of the serious growth inhibition and the viability loss of *B. napus* roots severe oxidative stress was observed: despite the elevated SOD activity $O_2^{\cdot-}$ accumulated, while the cells failed to eliminate the formed H_2O_2 because of the reduced APX activity. Moreover, a remarkable lipid peroxidation was visualized in the roots.

Long-term Zn excess caused oxidative and nitrosative stress in both species and despite their higher level in *B. juncea* root tips, it proved to be more tolerant according to the growth parameters.

Based on the morphological and physiological results, I conclude that *B. napus* tolerates Cu excess better than *B. juncea*. In contrast, *B. juncea* possesses elevated Zn tolerance compared to the other species. My results support the species-specificity of metal tolerance.

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Study of cuckoo-host relationships on a great reed warbler population in Hungary

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The brood parasitic common cuckoo (*Cuculus canorus*) lays its eggs to nests of other bird species, where the foster parents incubate, hatch and feed the cuckoo. A typical host species is the great reed warbler (*Acrocephalus arundinaceus*), breeds in wetland areas in Hungary, and builds open nest in reed beds. The modal clutch size of great reed warblers is 5 eggs and incubation time is about 11-12 days. We investigated several aspects of ecological relationships between common cuckoos and great reed warblers, including behavioural and evolutionary adaptations. However, we also applied microbiological and molecular methods.

In our first study, we examined bacterial loads on the eggshells of common cuckoos and great reed warblers. During our field work we collected samples from the eggshell surface of both cuckoo and great reed warbler eggs, either from parasitized and non-parazitized clutches to compare bacteria of the eggshells. We hypothesize that cuckoos, as nest visitors, may influence on the hygiene of nests of great reed warblers by changing bacteria loads. Previous studies showed that environmental factors, such as temperature and humidity, may affect bacterial loads on the eggshells in cavity nesting birds. We hypothesized that these environmental factors also affected the hygiene of open nests of great reed warblers. From these factors we measured ambient light conditions, both in the visible and UV spectra.

Keeping eggs dry in avian nests during the incubation period may reduce bacteria load on the eggshells, so it may protect the eggs from bacterial infections. A few previous studies have already showed the antimicrobial effects of incubation in cavity nesting birds, but, in the first time, we studied these effects under more variable environmental conditions, on an open-nesting bird species.

During the co-evolution arms race between common cuckoos and great reed warblers both the brood parasites and hosts developed ecological adaptations. The adaptations developed by the brood parasite help successful parasitism (e.g. "mimetic eggs"), but the adaptations by the hosts are against the brood parasites ("antiparasite adaptations", e.g. egg discrimination). We evaluated the changes of eggshell spottiness of common cuckoos and great reed warblers in time. Previously, we photographed parasitized clutches of host eggs held in museum collections (Natural History Museum, Tring, Mátra Museum, Gyöngyös, and Hungarian Natural History Museum, Budapest), and we also took digital photos during our field work. All eggs were collected from Hungary. We had four treatments from the years of 1900s, 1930s, 1960s, and 2000s. For analysing images we used ImageJ and Matlab programs. We wanted to reveal how spottiness changed in common cuckoos and great reed warblers. We analysed these changes by statistical pattern analysis on eggs from the last hundred years, focusing on cuckoo egg mimicry to host eggs.

The study was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

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Investigation of South-Indian *Fusarium* isolates from human keratitis

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The genus *Fusarium* is a large group of hyaline filamentous fungi. They are widely distributed in soil as harmless, saprophytic organisms. However, some members of this genus are capable of causing infection in plants, animals and humans. *Fusarium* spp. are the most frequently isolated causative agents of human keratomycosis in South India. Antifungal susceptibilities of different *Fusarium* species complexes (SCs) vary, and members of the *F. solani* SC (FSSC) show remarkable resistance to most clinically applied antifungal drugs. Thus the misidentification of the causative agent and the subsequent application of an inappropriate antifungal therapy could result in the loss of vision. Using molecular techniques in laboratory practice instead of conventional morphological methods can make the identification process more accurate and faster. New antifungals and alternative treatments would also be appropriate to prevent or treat the infection.

For these reasons, first we identified *Fusarium* strains isolated from human keratomycosis at the Aravind Eye Hospital and Postgraduate Institute of Ophthalmology (Coimbatore, India) in the years 2004-2005 and 2010-2011 using different molecular methods. We also examined the SC diversity between the two sampling periods. Our results indicate that the members of the FSSC are the most frequently isolated species from keratomycosis in South India, and the incidence of the less frequent human pathogenic *Fusarium* species seems to be increasing.

We also determined and compared the antifungal susceptibilities of the previously mentioned strains. Natamycin (NTM) proved to be the most effective drug against the tested isolates, followed by amphotericin B and terbinafine (TRB). Changes in the minimal inhibitory concentration (MIC) values of NTM and TRB were not observed between the isolates derived from the two sampling periods, but the *in vitro* susceptibility to azoles decreased up to 2011. NTM and TRB were also applied in antifungal combination susceptibility tests because of their high *in vitro* efficacy and their differing antifungal mechanisms. These compounds together showed a similar or a better antifungal activity on Fusaria than each of the compounds alone, as they could interact synergistically.

As a potential alternative cure for the infection, we examined the *in vitro* inhibitory effect of 9 different essential oils on 18 *Fusarium* strains isolated from keratitis. The lowest MICs were observed in the case of *Cinnamomum zeylanicum* oil; and its component, trans-cinnamaldehyde (tCA) was also tested and showed the same activity against the investigated isolates. The *in vitro* interaction between tCA and NTM was also determined. Furthermore, we investigated the antifungal mechanism of cinnamon oil and tCA by microscopic observations. Based on these observations both the oil and its component caused delayed or inhibited germination of conidia and reduced cellular metabolism. Thus, they can be potentially used in the treatment of *Fusarium* keratitis. However, the preliminary *in vitro* studies suggest that their simultaneous application with antifungal drugs, such as NTM, will not increase the efficacy of the therapy.

The investigation of phylogenetic relationships among clinical and environmental isolates and the production of extracellular enzymes, as potential virulence factors, are in progress.

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Up-regulation of defense genes in pepper leaves inoculated with tobamoviruses

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Virus infections result in substantial alterations of gene expression patterns in infected plant tissues including the up-regulation of a wide variety of defense-related genes. These defense reactions are controlled by a complex, multilayered regulatory network in which various transcription factors and defense-related plant hormones play critical roles. In addition, host intracellular membrane lipids also substantially influence virus replication. Upon infection, tobamoviruses induce substantial modifications in intracellular host membranes in order to create protected viral replication compartments. During this process the structure of membrane lipid bilayers is substantially modified. Viral RNA synthesis is highly sensitive to lipid composition and particularly to the level of unsaturated fatty acids.

In recent years our research has been focused on the defense reactions of pepper (*Capsicum annuum* L.) plants following virus inoculations. We have used two different viruses in order to compare compatible and incompatible pepper-virus interactions. Inoculation with *Obuda pepper virus* (ObPV) led to the appearance of hypersensitive necrotic lesions on the inoculated leaves. In contrast, very mild symptoms appeared on the leaves inoculated with *Pepper mild mottle virus* (PMMoV). Although these plants seem to be healthy, the virus is spreading from the infection site into the whole plant causing very serious stunting and the pepper fruits will be very strongly distorted.

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ObPV-inoculation resulted in the marked up-regulation of genes encoding PR-proteins, a patatin-like lipase (lipid acil hydrolase), a defensin, a 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and a dioxygenase participating in carotenoid degradation. In addition, ObPV-inoculation led to a rapid and massive up-regulation of several individual 9-lipoxygenase (*9-LOX*) genes. In contrast, *13-LOX* genes were only moderately induced by ObPV. The expression of several genes encoding WRKY transcription factors were also induced by ObPV. In contrast, the expression of defense genes increased in most cases to a lesser extent in PMMoV-inoculated, susceptible leaves or in mock-inoculated leaves. Plant hormones and an ethylene precursor (salicylic acid, methyl-jasmonate, and ACC) induced very differently the expression of individual *LOX* and *WRKY* genes.

In summary, our results showed that the rapid and massive up-regulation of defense genes encoding PR-proteins, LOXs and WRKY transcription factors in the incompatible pepper-ObPV interaction contributes to antiviral resistance. We suppose that by the rapid up-regulation of *9-LOX* genes pepper plants are able to alter the structure of intracellular membranes in order to inhibit the replication of invading tobamoviruses.

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Investigation of the different mechanisms of the innate immune response of *Drosophila melanogaster*

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Drosophila melanogaster has been widely used model organism to study host response to microbial and parasitic infections. The chitin cuticle of the adult *Drosophila* is the first barrier against microbial invasion. Injury of the cuticle activates hemolymph clotting, which blocks the loss of body fluids and the spreading of the microorganisms into the hemocoel by immobilizing bacteria at the wound site. Pathogens entering the hemocoel activate both cell-mediated and humoral immune responses. The cell-mediated arm of the immune response is carried out by the hemocytes, the production of antimicrobial peptides are regulated by the Toll and the immune deficiency (Imd) pathways.

We developed and validated a new method to identify novel factors involved in the hemolymph coagulation and in the host-pathogen interactions after septic injury.

The method, based on inducing lesion by removing the tarsal segments of the first pair of legs of *Drosophila* adults and exposing them to different bacteria, imitates injury that often occurs in the natural habitat. The technique was validated by using mutant variations of different components of the immune response; blood clotting as well as the involvement of a number of genes known to be instrumental in the humoral and cell-mediated immune responses of *Drosophila* was confirmed. We used the slightly pathogenic *E. coli*, the semi-pathogenic *B. cereus* and the highly pathogenic *S. marcescens* and monitored the viability of the flies. First, we tested the survival of the control w^{1118} and mutant flies after sterile injury and the survival of the non-injured w^{1118} and mutant lines (spz^{2}/spz^{4}, $Dredd^{EP1412}$, Rel^{20} and Hml^{f03374}) treated with *E. coli, B. cereus* and *S. marcescens.* We found that the survival of non-injured mutant flies treated with *E. coli, B. cereus* and *S. marcescens* were similar. The injury itself do not affect the survival of the animals, except for the Hml^{f03374} homozygotes, which lose more hemolymph after wounding and showed decreased survival rate following both sterile and septic injury compared to the control. We found that the Imd pathway mutants $Dredd^{EP1412}$ and Rel^{E20} and the hemolymph clotting factor *Hemolectin* (Hml^{f03374}) mutant flies showed reduced viability after either *B. cereus* or *E. coli* infection, while the *spätzle* (spz^{2}/spz^{4}), involved in the Toll pathway, was significantly sensitive to *B. cereus* infection. By using this novel method, we have found that the *raspberry* gene is involved in the survival of the fly after septic injury, since the mutants have decreased survival rate after *B. cereus* infection. This gene encodes the *Drosophila* inosine monophosphate dehydrogenase, and is a key enzyme of the *de novo* synthesis of guanine nucleotides. In mammals, *de novo* GMP synthesis is required for lymphocyte proliferation and in the immune response. We will study the function of the *raspberry* in the immune response of the *Drosophila*.

Our new method is suitable for high-scale screening of key factors involved in host-pathogen interactions following a septic injury. It also offers an alternative to previous experiments, where microinjection needle were used to administer microbes into the body cavity. A major advantage of this method is that the wound by itself is insignificant, the effect on survival can be attributed entirely to the infection and the defensive capabilities of the host organism.

Furthermore, we identified a new marker molecule 3A5 in the cytoplasm of a subset of plasmatocytes in all hematopoietic compartments, in the circulation, in the lymph gland and in the sessile tissue and in the hemolymph. We study the function of 3A5 molecule in the *Drosophila* immune response and in the coagulation reaction.

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Analysis of fungal fatty acids and prostaglandin-like compounds

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Candida parapsilosis is the third most frequently isolated *Candida* species in candidiases, especially in special patients groups such as low birth weight neonates, where *C. parapsilosis* even outmarks *C. albicans*. A number of biochemical parameters could influence the virulence of these fungal species, whose research is important to understand the mechanisms of whole infection process. According the previous results of our research group based on the transcriptome analysis of the *in vitro* host-pathogen interactions process, the prostaglandin- and the fatty acid pathways of *C. parapsilosis* could play an important role in the pathogenesis. Therefore, we aimed to develop a reliable measurement method for the analysis of metabolites, which are originated from the pathogenicity-linked biochemical pathways and are involved through the interactions as the final chemical effectors including prostaglandins and fatty acids.

In the first part of our work, we developed a liquid chromatographic method using fluorescence detector to analyse prostaglandins from the ferment broths of the wild type and UGA3 (a putative transcription factor, that can play a crucial role in fungal prostaglandin biosynthesis) mutant *C. parapsilosis* strains. After the optimization of sample preparation on solid phase, the evaporated extracts were derivatized to create fluorescence-active derivatives of prostaglandins. In this reaction the fluorescence molecule (Br-DMEQ) was linked to the carboxyl group of prostaglandins in the presence of aprotic solvent and catalyzer. The amounts and the ratios of the reaction components were optimized for the amount of the reaction products leading to the opportunity of the more sensitive measurement. The separation after the testing of several stationary- and mobile phases was carried out on the Phenomenex XB-C18 column with water/acetonitrile supplemented both with 0.1% acetic acid resulting the determination of seven prostaglandins. For the enlargement of the number of the detectable prostaglandins a mass spectrometric analysis was also developed in negative ESI ionization mode, which was able to determine 18 prostaglandin components. Furthermore, the fatty acid content of the wild type and a fatty acid desaturase deficient strain (OLE2) were analyzed with GC-FID technique developed for the analysis of 37 both of saturated and desaturated fatty acid methyl esters.

In the second part of our study, we dealed with the development of a rapid, high-throughput analytical method for the monitoring of the economically important fungal products, fatty acids, from the biomass of the *Mortierella* species to collect information about the effect of abiotic parameters of cultivation media to the production and the composition. In the method, the carboxyl groups of the extracted fatty acids were also tagged with Br-DMEQ and a short HPLC run was applied on core-shell chromatographic column to separate eleven fatty acids, which were in the scope of the study.

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Application of various imaging techniques for plant stress diagnostics

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Plant growth is affected by various factors. The resistance of the plant to withstand various biotic and abiotic stress factors plays a vital role for its growth and development. Our objectives were to combine various non-invasive imaging techniques (Digital imaging, Near- infrared (NIR) imaging, thermal imaging and chlorophyll fluorescence imaging) for studying stress responses induced by drought, chemical treatment and fungal pathogens in wheat seedlings (*Triticum* spp). Severe drought stress was induced by growing drought tolerant and drought-sensitive wheat genotypes subjected to decreased soil water content (10% field capacity as compared to 60% in the well watered control), while chemical stress induced by silica nanoparticles (10-20 nm particle size, 1000 mg/L) was studied in hydroponically grown wheat seedlings. Two week old near-isogenic wheat lines possessing various tan spot resistance genes were infected with pyrenophora tritici-repentis (PTR) fungal pathogen and characterized. OJIP fluorescence induction kinetics showed characteristic differences between cultivars in response to drought stress as there was an increase in variable fluorescence in response to SiO_2 NPs treatment. Gas exchange measurements showed lower net photosynthetic CO_2 uptake during drought stress, while CO_2 uptake was enhanced in response to SiO_2 NPs treatment. Thermal imaging indicated stomatal closure based on lower transpiration rate under drought stress, while increased evaporative cooling through the stomata was seen in response to SiO_2 NPs. Increasing drought stress activates photosynthetic electron transport rates in water stressed drought sensitive cv. However, we observed higher quantum yields of PSI and PSII photochemistry in SiO_2 NPs treated wheat seedlings. Chlorophyll fluorescence imaging has proven to be a promising tool for characterization and early detection of tan spot disease in wheat *in vivo*. NIR images were able to detect the loss of water content in the area of tan spot infection on various wheat cultivars.

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Genetic analysis of the cooperation between the *bxd* PRE and the neighboring embryonic enhancers

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During embryonic development, proliferating cells are getting committed to different cell fates to create different tissues. This process is regulated by epigenetic factors generating tissue specific gene expression profiles maintained during the life of a cell and transmitted to its descendants by modifications of higher order chromatin structure.

To study the process of epigenetic gene regulation, the homeotic bithorax-complex (BX-C) of Drosophila proved to be an excellent model-system. Subtle alterations of the chromatin structure of BX-C results in easily detectable segmental transformation. The three genes of BX-C are regulated by nine large, segment-specific *cis*-regulatory regions. The appropriate active or inactive conformation of these regulatory regions is maintained by the TRITHORAX or POLYCOMB group of proteins, binding to specific elements in the regulatory regions, called Trithorax- or Polycomb-Response-Elements (TRE or PRE), respectively.

Previously, we have analyzed a chromatin silencer, called PRE, in the *bithoraxoid* (*bxd*) *cis*-regulatory region of the *Ultrabithorax* (*Ubx*) homeotic gene. In the recent work we studied two embryonic enhancers, S1 and S2, straddling the *bxd* PRE. These enhancers have been identified in transgenic assays, but we wanted to reveal their role and the functioning in their natural chromosomal environment. For this purpose, the S1 and S2 enhancers were deleted using an advanced form of gene conversion developed by our group. We analyzed the mutant phenotypes in adults, as well as changes in gene expression patterns using immuno-histochemistry and native GFP fluorescence combined with high resolution confocal microscopy. In addition, we generated several other deletions, which removed additional regulatory elements in the *bxd* region. We found that S1 and S2 have significant roles in the initiation of the *bxd cis*-regulatory region. Our results also suggest that the S2 embryonic enhancer cooperates with the *bxd* PRE, but the mechanism of this cooperation is not fully understood yet. We try to explain the mechanism of this cooperation, hereby to answer how early initiators can affect chromatin structure and functioning of regulatory regions. We hope our experiments will contribute to the understanding of the general and the specific role of enhancer function.

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The role of an anionic lipid, the phosphatidylglycerol, in the cyanobacterial cellular processes

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Cyanobacteria are Gram negative photosynthetic bacteria.

Phospholipids play important role in the structure of cell membranes and actively participate in different membrane related cellular processes.

Cyanobacterial membranes contain four types of lipids, two neutral and two negatively charged lipid. The phosphatidylglycerol (PG) is the only phospholipid present in the cyanobacteria. It has been demonstrated that the PG plays important role in the structure formation and function of the photosynthetic complexes.

In case of PG deprivation, the *Synechocystis* sp. PCC6803 cells show enlarged cell volume and *Synechococcus* sp. PCC7942 elongated cell size. If the PG is re-added to the cultures, the normal cell morphology is recovered. This might suggest that the lack of PG affects the normal division process of the cyanobacterial cells.

Many cell division proteins have been identified mainly in *Escherichia coli* bacteria. The homologues of these proteins were found in cyanobacteria.

A determining step of bacterial cell division is the polymerization of the tubuline-like FtsZ protein in a ring like structure in the mid-cell region. The localization of the Z-ring is a highly regulated process. The regulation differs in Gram negative and Gram positive bacteria. The cyanobacteria possess proteins characteristic to Gram negative and Gram positive division along with cell division proteins unique to cyanobacteria or higher plant plastids. During the division process, a number of division proteins get in contact with the plasma membrane. Changes in the membrane composition might affect the cell division. Many studies suggest the importance of phospholipids in the division processes. In our studies, we follow the changes in cell division in a phospholipid-lacking live cyanobacterial system.

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Examination of *Trichoderma* strains isolated from the rhizosphere of vegetables for the purposes of developing environment-friendly in field technologies

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Organic farming is becoming nowadays more and more important in the agriculture. Organic farmlands are exposed to dangerous xenobiotics through distinct pollution drift effects such as wind-driven, pesticide-containing dusts and xenobiotic-containing rains. In order to achieve organic farming, there is a need for the development of new techniques which allow the bioremediation of lands previously used in common, intensive agricultural practice. Organic agriculture also faces the problem of pests including the damage caused by plant pathogenic fungi, therefore the implementation of biological control as a possible, environment-friendly solution is also of increasing importance.

Trichoderma strains were isolated from vegetable rhizosphere samples on dichloran-Rose Bengal medium. After purification of genomic DNA, the PCR amplification of the internal transcribed spacer (ITS1-5.8S rDNA-ITS2) region and its sequence analysis were used for the identification of the isolates at the species level. Altogether, 45 *Trichoderma* isolates were identified from the examined samples. The detected *Trichoderma* species were *T. asperellum*, *T. atroviride*, *T. citrinoviride*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. koningiopsis*/*T. ovalisporum*, *T. longibrachiatum*/*H. orientalis*, *T. pleuroticola* and *T. virens*.

In vitro antagonism of selected isolates was examined in dual culture tests and the Biocontrol Index (BCI) values were determined for the particular isolates. Certain *T. asperellum*, *T. virens* and *T. atroviride* isolates proved to possess good *in vitro* antagonistic activities against plant pathogenic *Fusarium solani, F. oxysporum, Phoma cucurbitacearum, Alternaria alternata, Botrytis cinerea, B. pseudocinerea* and *Rhizoctonia solani* strains.

Fungicide susceptibilities were measured by the microdilution method and the Minimum Inhibitory Concentration (MIC) values were recorded. Ten fungicides were tested in the concentration range of 512 µg/ml to 1 µg/ml. Strain *T. asperellum* SZMC 20866 showed resistance to 9 fungicides and was sensitive only to Maneb (MIC: 256 µg/ml). The *T. atroviride* strain SZMC 20781 showed similar fungicide resistance properties to those of *T. asperellum* SZMC 20866. MIC values of *T. harzianum* SZMC 20770 were 256, 512, 32, 64, 512 and 128 µg/ml for Cyproconazole, Fenarimol, Imazalil, Maneb, Penconazole and Thiram, respectively. The strain most sensitive to the tested fungicides was *T. virens* SZMC 20779.

The effect of temperature on growth in a range of 5 – 40 °C was also examined, and the water activity (a_w 0.997 – 0.922) and pH (2.2 – 8.0) dependence determined in the case of the isolated *Trichoderma* strains. Temperature values of 20-30 °C were optimal for the growth of *Trichoderma* strains, while none of the strains were able to grow at 5 °C. The examined strains were able to grow in a wide range of pH from 2.2 to 8.0, the maximal growth was observed under acidic conditions at pH 4.0. The highest tested a_w value (0.997) seemed to be optimal for the growth of all strains. Only limited growth was observed at 0.945 in the case of only three examined strains.

The results of the recent study suggest that the rhizosphere of vegetables may be a rich source of potential biocontrol agents for environment-friendly, organic agricultural production. We identified 3 *Trichoderma* strains which seem to be very promising for the development of microbial products with multiple beneficial effects for the purposes of organic farming.

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Physiological and molecular analysis of salt stress-induced PCD in tomato

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As saline soils and waters are common around the world, salinity is one of the major abiotic stress which largely limits plant growth and productivity. The ability of plants to tolerate salt stress is determined by multiple biochemical pathways; the most important is that the plant facilitates retention and/or acquisition of water, protects chloroplast functions, and maintains ion homeostasis. Severe salinity induces programmed cell death (PCD) in plants takes place in eukaryotic cells of different origin. One typical hallmark of PCD in plants is an increase in the process of protein degradation which is initiated by reactive oxygen species (ROS) and nitric oxid (NO) and involves the action of proteolytic enzymes. ROS and NO generation is one of the earliest response of plant cells under abiotic stresses. Protein degradation is probably the most important degradation process that occurs during PCD. The total protein content of tomato leaf gradually decreased with increasing concentration of NaCl. This decrease in protein content might be due to the increasing activity of cysteine- and serine proteases. For this reason, many of the genes up-regulated during PCD are proteases. The four major classes of proteases: cysteine, serine, aspartic acid and metalloproteases, exist in plant cells. Genes that encode proteases are activated by different ways. Expression of these genes that encode cysteine proteases has been shown to induced by environmental stress such as salinity. We studied different genes, for instance *MCA1, CYP, CP,* which encode various types of proteases participating in plant PCD. In addition, inhibitors encoding genes (*PI2* and *LTC*)

and BAX inhibitor-1 homolog gene (*BI1*) were also tested. According to our results in addition to the cysteine proteases their inhibitors also have fundamental role in the regulation of protein degradation. It is very important to see the connection between the processes of salt induced PCD and different stress hormones from which one of the most important is abscisic acid (ABA) which might have a role in this regulatory pathway. ABA is commonly recognized as naturally occurring plant hormone. ABA plays a key role in many developmental processes, from the promotion of seed desiccation tolerance to the synthesis of storage proteins and organ senescence. In addition, ABA acts as an endogenous messenger in the regulation of plant-water status and regulates some aspects of the plant's physiological responses to environmental stresses, such as osmotic stress-induced stomatal closure and salt, drought and cold tolerance. Our first results show that ABA might induce protease activity during PCD.

To gain a better understanding of the salinity stress responses at physiological and molecular level in cultivated tomato we carried out a comparative physiological analysis. Tomato has a medium tolerance to salinity and it can acclimate to high salinity at morphological and physiological level. In addition to the wild-type, an ABA deficient-tomato mutant, *flacca* was studied, too. Plants were treated with sublethal and lethal concentrations of NaCl. The growth of this plant is not inhibited by medium NaCl stress but it is affected by strong one. The salt stress-induced changes in ROS content and in the gene expression level were shown at the beginning of the treatment. Protein content and protease activity were also studied as a function of time. There was a nice correlation between decreased protein content and increased protease activity in the first 24 hours. Finally, we suggest that cysteine proteases might participate in salt-induced PCD in tomato as a function of time depending on intensity of the stress.

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Nuclear function for the actin binding cytoskeletal protein, moesin

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The most dynamic component of the cytoskeleton in every eukaryotic cell is the microfilament network of linear polymers of actin subunits. Extensive research in the past decade has significantly broadened our view about the role actin plays in the life of the cell and added novel aspects to actin research. The discovery of the existence of nuclear actin became evident only recently. Nuclear activities, including transcriptional activation in the case of all three RNA polymerases, export of certain mRNAs and proteins, chromatin remodeling, and nuclear assembly after mitosis, all depend on actin.

Moesin, the well-known cytoplasmic actin binding protein is the only member of the evolutionary conserved mammalian ezrin-radixin-moesin (ERM) protein family in *Drosophila melanogaster*. ERM proteins are responsible for the organization of the cortical actin network and anchor membrane proteins to it. They all have an N-terminal FERM domain, which is a general protein binding domain, a mid-domain which is a flexible hinge region and a C-terminal actin-binding domain. Our laboratory demonstrated previously that moesin is present in the interphase nucleus but the biological significance of this localisation remained unknown.

We are studying currently the exact localisation and function of moesin in the interphase nucleus. Our experiments showed that moesin accumulates as a ring at the nuclear envelope; it is present in the nucleoplasm, in some chromosome regions and occasionally in the nucleolus. We found that the quantity of moesin in the nucleus increases upon heat stress, which suggests a function for moesin in the nucleus and that its transportation into the nucleus is an active process.

To further analyse the chromosomal localisation of moesin, we performed immunostaining experiments on larval polytene chromosomes. Moesin was detected in the euchromatic bands moreover, it also showed colocalisation with the active form of RNA Polymerase II, and the intensity of the accumulation of the two proteins on the chromosomes was identical. Moesin staining was found especially strong in the chromosome puffs which are special euchromatic regions of extremely active transcription sites in the polytene chromosomes. The transcription on a transgene regulated by an inducible promoter resulted in the formation of an extra moesin band in the corresponding chromosome region suggesting that moesin is required for transcription rather than the formation of the puff structure. This idea was confirmed by the finding that the disassembly of the RNA polymerase complex caused by the drug triptolide, resulted in the detachment of moesin from the chromosomes.

We have also performed a preliminary screen to identify the proteins that are responsible for the nuclear transport of moesin. Our results both with cultured cells and in the live animal revealed that the Nup98 protein is involved in the nuclear export of moesin.

In summary, our results demonstrate that besides its cytoplasmic functions, moesin also plays important roles in the nucleus. We have shown that moesin is actively transported to the nucleus where it participates in the process of RNA transcription.

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Discovery of novel fluorochromes for use in plant studies

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Plant cells store neutral lipids such as triacylglycerols in distinct cytosolic organelles called oil bodies (OBs) (also referred to as lipid bodies/droplets, spherosomes or oleosomes) Although initially thought to be found only in oleogenic seeds and fruits, where they serve as fuel for the growth and development of seedlings prior to photosynthetic establishment, in recent years, studies have shown that OBs are quite ubiquitous. Their role extends from being static depots for carbon storage to stress response, lipid homeostasis, pathogen resistance, hormone metabolism and signaling and a specialized role in anther development. Plant OBs similar to their counterparts in yeast and mammals, are highly dynamic. Proteomic analyses have revealed that, there are a host of proteins which reside on the surface of these OBs and the exact content is changing under varying conditions. But a lot still remains to be uncovered in the area of OB protein and lipid composition as well as OB transport, mechanism of protein targeting, assembly and regulation. Live cell analysis is thus required to unravel the dynamic regulation of this important organelle.

Live-cell imaging offers a unique opportunity for investigating OB regulation. A large collection of imaging tools based on fluorescence is currently available. Varying colors of photostable genetically-encoded fluorescent proteins can be used in multiplexed tracking of protein remodeling on OBs. Photo-switchable fluorescent proteins as well as fluorescent timers allow quantitative assessment of OB protein dynamics. Fluorescent sensors, such as those based on fluorescence resonance energy transfer (FRET), can be applied to follow protein conformational changes or protein-protein interactions relevant to OBs. These tools, combined with the use of reliable OB markers, represent a versatile scheme for investigating OB biology. However, commercially available live cell dyes are limiting in their ability to penetrate cell walls and those that are permeable, in addition to other drawbacks such as photostability (BODIPY) and broad emission range (Nile Red), also restrict multicolor imaging, as most fluoresce in the green to red region of the visible spectrum.

In the present study, we report new fluorochromes as markers for OB in living plant cells. The fluorochromes, which are thalidomide analogs were in-house synthesized and were tested on live plant suspension cultured cells at various concentrations. The spectral emission range for the fluorochromes was identified and cell viability assays were also performed. We could also observe that the OBs remain highly mobile after staining with these fluorochromes, suggesting that the mobility dynamics were not affected significantly. We expect that these new chemicals will provide a novel approach for microscopy analyses of OBs in live plant cells.

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Bacterial symbionts enhance photo-fermentative hydrogen evolution of *Chlamydomonas* algae

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Chlamydomonas reinhardtii represents a well-established algae model system for biohydrogen generation. Two major methods are known for sustained *Chlamydomonas*-based hydrogen evolution. Hydrogen production using sulfur-deprived photoheterotrophic cultures of *Ch. reinhardtii* is the most widespread approach. Sulfur deprivation leads to complete anoxia via the inactivation of the photosynthetic system, thus, the extremely oxygen-sensitive algal Fe-hydrogenase will be able to become functional. Another algal hydrogen evolution approach is possible through dark fermentation. In dark and anaerobic conditions algae can catabolize endogenous carbohydrates or secondary metabolites generating organic acids, ethanol, CO_2 and H_2. The vast majority of algal hydrogen evolution studies were conducted using pure algae cultures. Our knowledge on the exploitability of algal-bacterial consortia in biohydrogen production is fairly short. Biohydrogen production capacity and growth rate of *Ch. reinhardtii* cc849 or the transgenic strain Iba co-cultured with *Bradyrhizobium japonicum* has been investigated. The sulfur-deprivation method together with *B. japonicum* inoculation resulted in enhanced rate of the oxygen consumption in the cultures, increased growth rate of algae and significantly improved hydrogen evolution rate. Our investigations aimed the elucidation of the nature and dynamics of algal hydrogen evolution observed in various algal-bacterial interactions.

The green algae *Chlamydomonas* sp. strain 549 was investigated for its hydrogen-evolution capability in algal-bacterial mixed cultures. Stable bacterial contaminations were identified during algae cultivation, the symbionts belonged to various genera, mostly *Brevundimonas* sp., *Rhodococcus* sp. and *Leifstonia* sp. All natural symbiotic partners enhanced fermentative algal hydrogen production. This phenomenon was not limited for the natural associations, increased algal hydrogen evolution was achieved by simple artificial algae-bacterium communities as well. Designed algal-bacterial co-cultures were tested in hydrogen evolution experiments, the highest hydrogen yield was obtained

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when hydrogenase-deficient *E. coli* was applied as symbiotic bacterium. The results showed that the oxygen elimination process is the most crucial factor for algal hydrogen production, efficient bacterial respiration is essential for the activation of algal Fe-hydrogenase.

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Identification of a novel effector cell type in the cell-mediated immunity of *Drosophila*, the multinucleated giant hemocyte

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Innate immunity is the first line immune defense against microbes, parasites and tumours which is composed of humoral and cell mediated events. In *Drosophila*, three main classes of blood cells, so called hemocytes, are the effector cells of cell mediated immunity. The plasmatocytes engulf microbes, produce extracellular matrix components, and provide systemic signals during microbial infections. Crystal cells contain crystallized prophenoloxidase enzyme, which is necessary for the melanization response. Lamellocytes arise upon immune induction, such as infestation by parasitoid wasps, and are required for the encapsulation reaction by forming a multilayered capsule around the parasitic wasp egg, which later melanizes. The effector hemocytes of the *Drosophila* larva originate from three hematopoietic compartments: the lymph gland which is a compact hematopoietic organ with multiple lobes, the sessile tissue where hemocytes are attached to the wall of the hemocoel, and the circulation. All three compartments contribute to differentiation of the effector hemocyte pool following immune induction.

The cell mediated immunity of *Drosophila melanogaster* is well studied; however, our knowledge on the immune response of other insects, in particular, other members of the *Drosophilidae* family is far from complete . The availability of various *Drosophila* species from different natural habitats allows to study the adaption of the cell mediated immune response to the different parasites. Recent studies show the diversity of the capsule forming cells of different Diptera species. According to these data the pseudopodocytes in *D. affinis* and *D. obscura* from the *obscura* group of *Drosophilidae* are capable of phagocytosis, similarly to plasmatocytes, however they are also involved in the capsule formation around foreign particles.

Our aim was to characterize the hemocyte subsets and the hematopoietic compartments in *Drosophila ananassae* from the *ananassae* subgroup. We identified a special giant hemocyte, which we named MGH (Multinuclear Giant Hemocyte) in *D. ananassae*, that appear after immune induction. To isolate different hemocyte subsets and to define their function, origin and formation, we produced monoclonal antibodies to subclasses of hemocytes and developed a transgenic reporter system which allows *in vivo* detection and manipulation of hemocytes and hematopoietic compartments in *D. ananassae.* As MGHs are similar to mammalian multinuclear giant cells, which play an important role in the formation of granulomas, we believe that *D. ananassae* could serve as a model for a better understanding of the development, structure and function of granulomas and of the multinucleated giant cells.

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Methodology of ancient DNA, and results to date

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Our research group isolates and studies ancient DNA (aDNA) from excavated human remains in collaboration with the Department of Anthropology. Sequence data obtained from ancient bones can unravel genetic relatedness of individuals, and populations. From a representative data set one can surmise population movements, and population history. The aDNA research can complement anthropological and archaeological data. For kinship studies routinly matrilinearly inherited mitochondrial DNA sequences, or patrilinearly inherited Y-chromosomal sequences are used, but autosomal loci correlated with known phenotypes can also be examined, such as monogenic disease genes, hair and skin color genes, or FOX2P gene, associated with speech ability.

In addition, aDNA of ancient pathogenes can also be obtained from their deceased carriers, which makes it possible to determine the distribution of prehistoric infectious diseases, such TB caused by *Mycobacterium tuberculosis.*

The preservation of aDNA, largely depends on the environment, and even under best conditions, it is largely degraded and fragmented. Usually trace amounts of 50-200 bp long DNA fragments are left, so classical methods apply PCR amplification, and cloning. The risk of contamination with modern DNA is very high, therefore special sterile laboratories are required for aDNA work. In the last few years the

appearance of new generation sequencing techniques opened a new dimension of ancient DNA studies, since from traces of DNA, large amount of sequence data can be obtained with this method.

We heve recently created a special sterile aDNA laboratory at the Department of Genetics. This so called pre-PCR laboratory is supplemented with a post-PCR, standard molecular laboratory in a distant part of the building (a requirement to prevent contamination). Both laboratories are equipped, and we have optimized DNA extaraction and amplification. In the pre-PCR laboratory, a simple method was adapted for bone's milling. For DNA extraction we also adapted a cheap but reliable modified silica powder affinity purification method. For DNA amplification we are testing various enzyme brands and conditions recommended by the manufacturer.

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Effect of hypoxia on MCF-7 cells' transcriptome and metabolic activities

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The hypoxic condition is prevalent in solid tumours and it is often associated with poor prognosis. Metabolic alterations that make possible for the cancer cells to survive and thrive under hypoxic condition are subject to high interest, however a systems-level understanding is still missing.

In order to emulate the hypoxic state, cell of a well established breast cancer model cell line (MCF-7) were cultured under normal oxygen concentration and subsequently exposed to hypoxia. To detect the cells' response to hypoxia, RNA samples were collected and sequenced from both conditions and mRNA abundances were determined.

With the aim of inferring the metabolic routes that may play important roles in the cancer cells' response to hypoxia, we employed the iMAT method that integrates gene expression data and a human genome-scale metabolic network reconstruction to predict metabolic reactions that are specifically altered in hypoxic condition. Beside, to gain a more global view of the functional changes underlying the hypoxia-response, we carried out a Gene Ontology analysis on the RNASeq data. In addition, to generally assess the predictive capability of the human metabolic network model, we applied an essentiality analysis and compared predictions to available high-throughput data.

The analyses resulted in the identification of 33 metabolic reactions which are specifically activated under hypoxia. The the majority of the detected reactions is distributed across 4 modules of cellular metabolism, namely sphingolipid metabolism, pyruvate metabolism, nucleotides metabolism, inositol phosphate metabolism. In addition, C160 fatty acid activation, diacylglycerol phosphate kinase and the arginine/lysine transporter were predicted to be active.

The predicted arginine transported and the reactions of the pyruvate metabolism will be subject to further experimental investigations by our collaborators in order to assess their role in hypoxia.

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Methane inhalation prevents from the quantitative changes in nitrergic myenteric neurons and intestinal motility disorders in a rat model of intestinal ischemic-reperfusion injury

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The gastrointestinal tract is highly susceptible to hypoxia, thus local or systemic circulatory disturbances are often associated with intestinal inflammation and enteric neuropathy. Inflammatory mediators influence the activity of enteric neurons, therefore, development of the intestinal inflammation is frequently associated with gut motility disturbances. Previously, we have demonstrated the anti-inflammatory effects of exogenous methane inhalation after IR. However, the effects of inhaled methane on the IR-related quantitative changes of enteric neurons or on the myoelectrical activity of the gastrointestinal tract were not investigated until now. Therefore, the main focus of this study was to investigate the consequences of intestinal IR and normoxic methane inhalation on the quantitative parameters of myenteric neurons and intestinal motility.

For the study 300-350 g male Sprague-Dawley rats were divided into three groups, these are: sham-operated, IR and methane-treated IR (n=8-8). Ischemia was induced by the occlusion of superior mesenteric artery. The inhalation of normoxic artificial air with 2.2% methane

was applied in the last five minutes of ischemia and first ten minutes during reperfusion. After anaesthesia the myoelectric activity of the gastrointestinal tract was monitored during ischemia (50 minutes) and reperfusion (120 minutes). Samples of the duodenum, ileum and colon were collected at the end of reperfusion phase. After an overnight fixation whole-mount preparations were prepared for immunhistochemical (HuC/HuD, nNOS and eNOS) staining. Biopsies from the small intestine were collected for biochemical studies. Tissue superoxide levels, xanthine oxidoreductase activity were determined to monitor the oxidative stress, and tissue nitrite/nitrate and nitrotyrosine levels were determined to study the levels of nitrosative stress.

At the beginning of ischemia the myoelectric activity sharply increased, then decreased gradually until the end of the reperfusion period. After methane inhalation a post-ischemic peak appeared in myoelectric activity at the beginning of the reperfusion period which then declined sharply and reached near the control level by the end of the reperfusion period.

After IR the total number of myenteric neurons did not change, but the density of nNOS and eNOS-positive myenteric neurons increased. Increase of the nNOS-immunreactive neurons in the duodenum were significant. After methane inhalation the density of the nitrergic myenteric neurons was similar to the neuronal density found in sham-operated rats. During IR the levels of tissue nitrite/nitrate, nitrotyrosine, and xanthine oxidoreductase activity increased significantly, while the methane inhalation prevented the intestinal tissues from the increase of oxidative and nitrosative stress markers.

Based on these results we hypothesize that due to the increased density of nitrergic myenteric neurons in IR the descending inhibition of intestinal peristalsis was enhanced. At the same time methane inhalation in the early stages of reperfusion prevented from the increase in the number of nitrergic myenteric neurons and the intestinal motility disorders.

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Development of a novel, somatic gene transfer system in the mouse

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Cancer is the leading cause of death in the developed world. Tumorigenesis requires the acquisition of mutations in proto-oncogenes and tumor supressor genes. Such genetic changes can be caused by mutagenic agents, chromosomal translocations or the disruption of the balance in epigenetic networks. A class of mutations, called „driver" mutations, affect a relatively limited number of genes that are functionally related to the key attributes of cancer cells. Contrary to driver mutations, „mutator" mutations act as enhancers of the tumorigenic process. According to the mutator hypothesis, mutator mutations decrease genome stability and, hence, accelerate the accumulation of random mutations, including those in proto-oncogenes and tumor suppressor genes. Our aim is to create a novel, somatic genetransfer system for the identification of candidate genes involve in the enhancement of tumorigenesis through the over-expression of native/mutant coding sequences or gene silencing with artificial miRNAs.

Type 1 tyrosinemia is a liver-based *metabolic disorder* caused by a deficiency of the enzyme fumarylacetoacetate hydrolase (Fah). The mouse model of this disease (Fah knock-out strain [Fah -/-]) offers the possibility to develop the new transgenic system. The primary treatment for type 1 tyrosinemia is nitisinone (NTBC). This drug prevents the formation of fumarylacetoacetic acid, which has the potential to be converted to succinyl acetone, a toxin that damages hepatocytes. Consequently, liver degeneration occurs due to the withdrawal of NTBC. However, the high regenerative capacity of this organ can be utilized to establish a new, healthy liver: wild type hepatocytes (Fah +/+) can migrate to the diseased organ and repopulate that within a few months after cell transplantation into the spleen. Thus, a Fah +/+ transgenic liver can be obtained from a genetically engineered hepatocyte pool in a Fah -/- recipient mouse.

Liver repopulation can be monitorised with a fluoresence marker gene that also serves the expression of artificial miRNAs, in addition to its indicator role. Furthermore, this somatic gene transfer system is adaptable for library screens due to the large amount of hepatocytes potentially involved in repopulation, resulting in the possibility to express multiple transgenes. Considering the somatic nature of the system, the classical method for generating transgenic mice can be avoided, and the number of experimental animals reduced. These adventages make this new practice faster and more cost effective. We hope that our technique for producing transgenic liver will become a valuable tool for cancer genetics.

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Anaerobic biodegradation of cellulose-rich substrates

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Depletion of fossil fuels and increase of global climate changes demand the usage of renewable energy sources. Biogas forms anaerobically during the decomposition of different organic materials. In the process, three syntrophic groups of microbes work together. These are the polymer degraders, acetogens and methanogens. The main components of the produced biogas are methane (55-70%) and carbon-dioxide (30-45%). After upgrading, biogas could be injected into natural gas grid. For biogas production, many waste and raw material is suitable. Plant biomass is the largest amount of biomass on Earth. Plants can harvest solar energy during photosynthesis and convert it to plant tissues, therefore have vast energy potential. Plant tissues consist of lignocellulose as the major component. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose is a recalcitrant complex polymeric carbohydrate, cellulases are needed for its efficient decomposition. Cellulases are divided into three major groups: endoglucanases (EC 3.2.1.4), exoglucanases (3.2.1.91) and β-glucosidases (3.2.1.21). Endoglucanases cut at random internal sites into the cellulose polysaccharide chain, generating new chain ends. Exoglucanases act on the reducing or nonreducing ends of cellulose, liberating glucose or cellobiose units. β-glucosidases hydrolyze cellodextrins and cellobiose to glucose which can be used in metabolic pathways.

For the utilization of substrates having high cellulose content – without pretreatment - the biogas producing microbial community should contain a significant number of cellulose producing bacteria and they should break down cellulose to easily utilizable sugar monomers. An adaptation strategy to adapt the community to lignocellulosic substrate has been developed. The experiments were carried out under thermophilic conditions at 55 °C. α-cellulose was used as substrate for the adaptation and the control fermentors received glucose as carbon and energy source. The changes in the concentration of volatile fatty acids were followed by HPLC, the β-glucosidase enzyme activity was monitored regularly. From the adapted microbial community, cellulose degraders were isolated and were also used as inoculum in the next set of biogas experiments. The cellulose degrading microbes had positive effect, elevated the biogas and methane yield. DNA was purified from the cellulose degrading consortia and was undergone metagenome analysis. In the thermophilic cellulose degrading consortium, the main orders were Thermoanaerobacterales (70%) and Clostridiales (10%). *Thermoanaerobacterium thermosaccharolyticum*, *Caldanaerobacter subterraneus*, *Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum* were identified as dominant strains.

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Uniform or different? Heterogeneity of murine bone marrow mesenchymal stem cells in differentiation and immunosuppression

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Bone marrow mesenchymal stem (BMMSCs) are adherent, colony-forming cells and are defined as multipotent cells differentiating into several cell types (e.g. osteoblasts, chondrocytes and adipocytes). BMMSCs have been found therapeutically beneficent in models for numerous human diseases by multiple processes including enhancement of tissue regeneration, supporting angiogenesis, subduing inflammation and modulating the immune response at the site of tissue damage. Despite the incessantly increasing number of preclinical and clinical MSC studies, there are some basic issues about MSCs, which still remain unresolved. The heterogeneity in differentiation potential of MSCs was demonstrated decades ago. Up to this day, few and inconsistent data have been collected reporting uniform or different immunosuppressive properties of single MSC clones, even if it is highly relevant to the therapeutical effectivity of MSCs.

We aimed to examine the heterogeneity of murine BMMSC population through characterizing 6 single cell-derived MSC clones (MSC1-MSC6) in terms of differentiation potential, support of angiogenesis and immunmodulation.

To examine whether MSC clones maintain the multipotency of BMMSC population, MSC clones were induced to differentiate *in vitro* into adipocytes and osteoblasts. While MSC2-6 differentiated into both lineages, MSC1 differentiated only into adipocytes.

Analysis of the ability of the MSC clones to support angiogenesis has been carried out using an *in vitro* model, the capillary mimicry assay. MSC clones were co-cultured with H5V endothelial cells and the capillary-like structures were evaluated. Whereas neither MSCs nor H5V formed capillary-like structures alone, all MSC clones supported similarly the development of these structures when co-cultured with H5V.

The *in vitro* immunmodulatory properties of BMMSC clones were compared in ConA-stimulated T cell proliferation assay. MSCs were co-cultured with T cells isolated from mouse lymph nodes in the presence of ConA and cell division of CFSE (a fluorescent dye used for proliferation assays) labeled T cells was followed by flow cytometry. All MSC clones inhibited significantly but not uniformly the T cell proliferation in the following order: MSC2>MSC4=MSC5>MSC1>MSC3>MSC6. Differences in the inhibition of T cell proliferation

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were reflected in expression level of *Nos2, Ptgs2*, the most important genes responsible for murine MSC-mediated immunosuppression. The strongest inhibitor MSCs expressed the most and the least inhibitor clones expressed the lowest level of these factors at mRNA level. Normally, MSCs exert immunosuppression at the site of inflammation, therefore the immunmodulation of MSC clones were tested in inflammation-mimicking milieu, treating MSC clones with pro-inflammatory cytokines, IFN-γ and TNF-α. Treatment of the cells with these cytokines resulted in upregulation of *Nos2* and *Ptgs2* gene expression in each MSC clone, and as a consequence, their inhibitory effect on T cell proliferation elevated. To find out whether MSC clones can exert immunmodulation *in vivo,* the effect of the most and the least immunosuppressive MSC2 and MSC6 clones, respectively, were tested in ovalbumin-induced delayed-type hypersensitivity response in mice. Intraperitoneal administration of MSC2 cells simultaneously with ovalbumin immunization significantly reduced, whereas MSC6 didn't change the ovalbumin-induced increase of footpad thickness, unless MSC6 cells were pretreated with IFN-γ and TNF-α prior to injection, in that case MSC6 also decreased footpad thickness increment vigorously.

Based on our results, we suggest that murine BMMSC population is homogenous in differentiation and angiogenesis support while heterogeneous in immunosuppression. Dissimilarity in the immunosuppressive function likely depends on the activation state of single MSC cells, since placing the cells into an inflammatory milieu, the immunomodulatory effect of different MSC clones becomes similar.

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Genetic analysis of *Saccharomyces cerevisiae RAD5* gene

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The sequence of DNA contains important information for the life of cells. Any damage of DNA leads to inaccurate function of the cell, or occasionally to its death. Therefore, proteins of DNA repair have a critical role in preserving the initial state of DNA.

At DNA damage, the replication polymerase stalls and the complex of Rad6 and Rad18 proteins ubiquitylates the Proliferating Cell Nuclear Antigen (PCNA), the processivity factor of replication polymerases, at its lysine 164 residue. Subsequently, the monoubiquitylated PCNA is polyubiquitylated by the protein complex of Rad5, Mms2 and Ubc13.

The Rad5 has three domains: RING, Helicase/ATPase and Hiran domain. While the RING domain has E3 ubiqutine ligase activity and the Helicase/ATPase domain has a replication fork reversal activity facilitating the formation of a chicken-foot DNA structure, the function of the Hiran domain is unknown despite of its predicted DNA binding capability.

To explore the particular functions of these Rad5 domains we tested the sensitivity of *ring* (CC914,917AA), *atpase* (DE681,682AA) and *ring-atpase* double point mutant strains with different mutagenic agents such as UV-light, methyl methanesulphonate and nitrogen-mustard.

We have found that the single mutant strains (*ring*, *atpase*) were sensitive to all tested mutagens and the double mutant strain (*ring-atpase*) had a higher sensitivity than the single mutants. We concluded that the ubiquitin ligase and the ATPase activities of Rad5 are not epistatic. This implies that these two activities have independent functions, but it is not exclude the existence of a common function. We also intended to investigate the relationship of these two activities of Rad5 with *RAD18* and *RAD51* DNA repair pathways. Although in our previous results epistatic relationship was not manifested among *RAD5*, *RAD18* and *RAD51* genes with none of the tested mutagens, one could not exclude the possibility that either the ligase or the ATPase activity of Rad5 could interact with one of these pathways. To explore this possibility the epistatic relationship of *ring* and *atpase* mutants was analyzed on *rad18*Δ and on *rad51*Δ background. On these deletion backgrounds point mutants represented the similar sensitivity like on wild type background. These results suggest that domains of Rad5 could function in the same DNA repair pathway with both of the proteins Rad18 and Rad51. Or probably none of the domains function with these two proteins, only they function with one or more other proteins out of Rad18 and Rad51. This hypothesis was confirmed by the higher sensitivity of *rad5*Δ*/rad18*Δ*/rad51*Δ triple mutant strain than *rad5*Δ*/rad18*Δ, *rad18*Δ*/rad51*Δ and *rad18*Δ*/rad51*Δ double mutants. Although this sensitivity could be caused by other functions of the Rad5 (e.g. function of Hiran domain). To prove this theory we intended to test the sensitivity of the point mutants on *rad18*Δ*/rad51*Δ background.

To explore the role of the Hiran domain, we generated mutations in its conserved regions. Five from the twelve mutant strains showed sensitivity to DNA damaging agents (nitrogen-mustard and hydroxyurea). Two mutant strains from the five showed the same growth curve like wild type on mutagenic treatment if over-expression of proteins were induced. It means the low expression level of these two mutant proteins caused their sensitivity in our previous experiments. The other three were overexpressed, purified and tested *in vitro* in biochemical assays. The LI265,266RR mutant protein exhibited wild type activity while the GA177,178RR and the G183R mutants showed no activity neither in helicase nor in ubiquitin ligase assays. We concluded that the GA177,178 and the G183 parts of Hiran domain are likely to have a basic role in both of the two functions of Rad5. Nevertheless it is possible that these mutations modify the whole structure of the protein and it loses all of its activities. To answer this question more structural studies are needed with both wild type and mutant proteins.

We concluded that the role of Rad5 out of the *RAD18* pathway is none or just partially related to *RAD51*. In addition, the ubiquitin ligase and the ATPase/helicase activities of Rad5 have independent function from each other, and these functions are not exclusively func-

tion with Rad18 or Rad51. Probably these independent functions act with one or more other proteins out of Rad18 and Rad51. Thus we expect that Rad5 has a more complex protein interaction network than it was previously known.

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Heterologous protein expression and *in vitro* analysis of *Drosophila melanogaster* proteins involved in telomere maintenance

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Our laboratory is particularly interested in the maintenance of genome integrity and telomere structure in *Drosophila melanogaster*. In eukaryotic cells, telomere prevents the chromosome ends from being detected as DNA double strand breaks and protects the coding regions from degradation. Telomere "capping" by the multi-subunit complex Shelterin, expressed in higher eukaryotes, averts the triggering of the DNA damage signaling pathways. In *Drosophila* this capping process is performed by a putative complex called Terminin, which is believed to have HOAP, HipHop, Ver and DTL (or Moi) proteins as subunits and it binds to the DNA in a sequence-independent manner. HP1 is generally regarded as the fifth subunit of the putative complex, thought it is not a strictly terminin-specific protein. HP1 is evolutionary highly conserved and plays a role also at non-telomeric regions while Terminin proteins manifest an accelerated rate of evolution and localize only at chromosome ends. Deletion of any of these genes results in telomere fusions. The physical interactions among Terminin proteins have been demonstrated *in vitro*. All these data support the existence of the Terminin complex.

The aim of my research was to study in details the putative Terminin complex and its suggested components by bioinformatics and molecular biological methods.

In silico analysis on the proportion of synonym and non-synonym codon substitutions confirmed that the full length HOAP, HipHop, DTL and Ver molecules have accelerated evolution compared to HP1. Interestingly, specific protein domains showed different rates of evolution and some of the hyper-variable domains have a role in protein-protein interactions.

The cDNA of each protein was cloned and expressed both in bacterial and in baculoviral expression systems. Our results indicated that DTL and Ver proteins form inclusion bodies in bacteria. Co-expression with at least two interacting partners resulted in soluble DTL and Ver proteins. A polycistronic construct containing all the five cDNA was engineered, and the purification of the complex is in progress. Early data suggest the formation of several sub-complexes rather than the assembly of a holo-complex in bacteria. The DTL protein produced in baculovirus system was applied in far-western experiments and although we were unable to detect interactions with Terminin proteins by this method, an interaction with a nuclear protein has been revealed.

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Biological control of plant pathogenic fungi by use of a *Bacillus amyloliquefaciens* strain

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The damage in agricultural production and storage by phytopathogenic fungi is a serious problem in agriculture. Biological control is an alternative method against phytopathogenic microbes. An organism, which can interfere with pests or pathogen species, is referred to as a biological control agent. A biological control agent can compete for niche and nutrients in the rhizosphere, inhibit the growth of plant pathogens by the production of antibiotics and extracellular lytic enzymes and act indirectly, promoting plant growth and triggering defensive systems of plants against pathogens. It is beneficial if antagonistic strains used for protection against pathogenic microorganisms are resistant to metals present in the soil. The antagonistic microorganisms have to grow and reproduce as well as produce antibiotics and extracellular enzymes in the presence of different metals. The use of biological control may not always be sufficient against pathogenic species. In this case, we need to use combined methods such as use biological control agents with pesticides and appropriate cultivation methods. The biocontrol agent needs to tolerate against the pesticides used in combined treatments.

The *Bacillus* genus contains various species with potential biocontrol capabilities. *Bacillus amyloliquefaciens* SZMC 22206 has been isolated and studied as a potential biocontrol agent in our laboratory. Our aims were to investigate the effect of metals and pesticides on the growth, the extracellular chymotrypsin-like enzymes and the antibiotics produced by *B. amyloliquefaciens* SZMC 22206 strain and to

perform antagonisms test against plant pathogenic fungi.

Using chromogenic substrates we investigated the secretion of protease, chitinase, cellulase and lipase enzyme systems of the *B. amyloliquefaciens* SZMC 22206 strain. The chymotrypsin-like protease activity was significantly high, so in our further studies we analysed this enzyme activity. The effects of metals and pesticides on the growth and chymotrypsin-like protease activity were also investigated. The effects of metals were analyzed with 0,1 mM, 0,5 mM and 1 mM concentration of copper, manganese, nickel, iron and zinc. The manganese did not inhibit the growth and chymotrypsin-like protease activity in the analysed concentrations. The other metals inhibited the growth and enzyme activity at 0,5 mM concentration. We analysed the effects of four pesticides (2,4-dicholorophenoxyacetic acid, carbendazim, linuron and chlortoluron) on the growth and the extracellular chymotrypsin-like enzymes of *B. amyloliquefaciens* SZMC 22206. These results indicated that both bacterial growth and the tested exoenzyme activities were significantly reduced in the presence of these pesticides. These findings suggest that the presence of chemical pesticides (e.g. in agricultural soils) can strongly affect the behavior and effectiveness of the non-target biocontrol bacterial species. *B. amyloliquefaciens* SZMC 22206 strain can produce fengycin, that is a cyclic lipopeptide antibiotic. The production of fengycin is increased if glicerol was used as carbon source, and if aspartic acid, ornithin or alanine were used as nitrogen sources. During in vitro tests the strain inhibited the growth of plant pathogen fungi like *Fusarium solani*, *Phoma cucurbitacearum*, *Phytophtora infestans*, *Alternaria solani* and *Botrytis cinerea*. On the basis of our results *B. amyloliquefaciens* SZMC 22206 shows the potential of being a promising biological control agent against plant pathogenic fungi.

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