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DISSERTATION SUMMARIES

The role of the orange carotenoid protein (OCP) and PsbU subunit in photoinhibition of Photosystem II in cyanobacteria

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Excess light is harmful for photosynthetic organisms. In *Synechocystis* 6803, high intensities of blue-green light induce Photosystem II fluorescence quenching which is specifically associated with a photoprotective phycobilisome related energy-dissipation mechanism. A soluble orange carotenoid protein (OCP), of previously unknown function, plays an essential role in this process (Wilson et al. 2006). In the absence of the OCP, the non-photochemical quenching (NPQ) induced by strong blue-green light in *Synechocystis* PCC 6803 cells is completely inhibited and the cells are more sensitive to high light intensities. In iron-starved *Synechocystis* 6803 cells a larger OCP-phycobilisome-related NPQ is observed in association with a higher concentration of OCP (Wilson et al. 2007). Highly conserved homologues of the OCP are found in almost all genomes of all cyanobacteria for which genomic data is available with the exception of *Synechoccocus elongatus*, *Thermosynechoccocus elongatus* and the *Prochlorococci*.

The existence of a blue-green light induced NPQ mechanism was tested in iron-containing and iron-depleted cells of *A. maxima* strain, containing OCP genes, and *T. elongatus* and *S. elongatus* PCC 7942 strains lacking the entire OCP gene. The kinetics of fluorescence changes was monitored by a modulated PAM (pulse-amplitude-modulated) fluorometer. Exposure of low blue light–adapted cells to high blue-green light intensities induced a quenching of the Fm' in OCP containing *A. maxima* strain. In contrast, illumination of OCP lacking *S. elongatus* and *T. elongatus* by strong blue-green light did not induce any decrease of Fm' indicating that in the absence of whole OCP gene this kind of photoprotective quenching cannot be induced. We tested possible relationship between the iron starvation and the blue-green light–induced NPQ.

Results obtained from fluorescence traces of 10 days iron-starved cells of *A. maxima* demonstrates increase of fluorescence quenching in compare with iron containing cells. This increase, like in a case of *Synechocystis* cells, can be related to larger accumulation of OCP under iron starvation. In contrast, exposure of prolonged iron starved *T. elongatus* and *S. elongatus* PCC 7942 to high intensities of light didn't induce any NPQ. Iron starvation induces the synthesis of the "chlorophyll-binding-iron-stressed induced protein", IsiA in all strains. The presence of IsiA causes a blue-shift in the room temperature Chl *a* absorbance peak (680-673 nm). Under iron stress conditions both chlorophyll and phycobilisome level decreased in all cells. According to the absorbance spectra iron starved *T. elongatus* and *S. elongatus* PCC 7942 cells show different reorganisation of the pigments compared to OCP containing *A.maxima and Synechocystis* cells: phycobiliprotein content decreased faster than chlorophyll content. Increase at 685 nm in fluorescence spectra generated at 77K by 430 nm in iron-starved *A. maxima* cells was attributed to the accumulation of uncoupled phycobilisomes with high fluorescence emission as previously shown in iron-starved *Synechocystis* cells. In contrast, in *T elongatus* and *S. elongatus* PCC 7942 cells the 685 nm emission (and 660 nm emission) decreased with the time instead of increase like in *A.maxima* cells. Thus, in *T elongatus* and *S. elongatus* PCC 7942 cells phycobiliprotein content decreased faster than chlorophyll content. This is probably essential for the longer survival of the cells in the absence of the photoprotective blue-green light induced NPQ mechanism (Boulay et al. 2008).

Photosystem II contains trans-membrane protein subunits and extrinsic proteins associated with the lumenal side. While great progress has been made in the understanding of the function of the water splitting apparatus, the role of the smaller subunits is still not well defined. Studies of deletion mutants demonstrated the importance of these proteins in stabilising the Mn cluster. PsbU is one of the extrinsic proteins which form the oxygen-evolving complex of PS II in cyanobacteria (Burnap et al. 1992). In this study we investigated the function of PsbU using a deletion mutant of the *psbU* gene in *S. elongatus* PCC 7942 (Balint et al. 2006). Flash-induced chlorophyll fluorescence measurements were used to monitor forward electron transfer at the acceptor side of PSII, as well as charge recombination processes of the reduced acceptors with oxidized donor side components. While forward electron transfer was similar between the wild type and the PsbU strains, a marked difference in the rate of back electron transfer in the presence of electron transfer inhibitor DCMU was observed in the mutant.

The overall fluorescence relaxation kinetics in PsbU mutant in the presence of DCMU resulted in a slow phase of 1.2 s and 82% amplitude whereas in mutant the corresponding values were 4.8 s, 64%. Moreover, thermoluminescence measurements have demonstrated that in the presence and absence of DCMU, the TL intensity in mutant cells was significantly increased, and this was accompanied by the shift of the peak position to higher temperatures for both the Q- and B-bands. Also the high light induced loss of oxygen-evolving activity in the presence and absence of an inhibitor of protein synthesis lyncomicin was monitored in the PsbU and wild type cells. When the high light illumination was performed in the presence of the protein synthesis inhibitor lincomycin the activity loss was significantly higher in mutant cells than in wild type. On the basis of these results, we conclude that PsbU is crutial for the stable architecture of the PSII.

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The expression of ABCC4 and ABCG2 xenobiotic transporters during keratinocyte proliferation/differentiation and in psoriasis

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Xenobiotic transporters are members of the ATP binding cassette (ABC) superfamily of proteins, responsible for the energy dependent transport of a broad range of chemically and structurally different compounds thus provide chemoresistance for various tumors. However, they also play a very important role in maintaining the chemical barrier function of organs such as brain, liver and gut (see for review Leslie et al. 2004). The human epidermis is one of the largest physical and biochemical barrier of the body. There have been only a few studies conducted regarding xenobiotic transporter expression in normal human keratinocytes and in human skin (Baron et al. 2001; Kielar et al. 2003).

We aimed to study the expression of eight xenobiotic transporters: ABCB1, ABCC1-6 and ABCG2 in in vitro models of keratinocyte differentiation. Terminal differentiation of normal human keratinocytes was promoted by increasing Ca^{2+} concentration. Validation of the differentiation model was achieved by the detection of proliferation markers Ki67 and integrin alpha 5 and differentiation markers keratin 1 and involucrin. The chemical-free synchronization of the immortalized keratinocyte cell line, HaCaT was used as another model (Pivarcsi et al. 2001), in which contact inhibiton and serum starving forces the cells into a highly differentiated quiescent state. Releasing HaCaT keratinocytes from cell quiescence by passaging and serum re-addition initiate redifferentiation and the cells start to proliferate synchronously.

Among the transporter genes tested ABCC4 and ABCG2 showed a proliferation associated expression in both *in vitro* models. ABCC4 and ABCG2 were highly expressed in undifferentiated, proliferating keratinocytes and their mRNA levels decreased in parallel with differentiation. ABCC4 and ABCG2 transporter protein levels also showed a decrease in differentiating keratinocytes, as revealed by Western blot and immunocytochemistry. Similarly, induction of ABCC4 and ABCG2 mRNAs and proteins were observed in synchronized HaCaT keratinocytes after release from cell quiescence, which further supported that ABCC4 and ABCG2 transporters have a possible function in proliferating keratinocytes.

ABCC4 protein was overexpressed in the basal layers of psoriatic lesional epidermis, supporting our *in vitro* results, while in keratinocytes of normal and non-involved skin it was expressed at very low levels. ABCC4 transporter may contribute to the pathogenesis of psoriasis since antiapoptotic/proliferation related cyclic nucleotides and important inflammatory mediators are ABCC4 substrates. ABCG2 transporter was expressed in normal and psoriatic non-involved epidermis and its expression was restricted to basal layer keratinocytes. Increased levels of ABCG2 protein was detected in psoriatic lesions, however its highest level of expression was observed in keratinocytes in the abnormally differentiating granular layer. It is known that human epidermis is a constitutively hypoxic tissue, which is more pronounced in psoriatic lesions. Hypoxia induces the generation of harmful porphyrins that are substrates of ABCG2, thus ABCG2 may protect keratinocytes from the accumulation of these compounds. The upregulation of ABCC4 and ABCG2 xenobiotic transporters in psoriatic lesions could significantly modulate drug distribution and effectiveness in the skin.

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Characterization of the novel opioid and nociceptin peptides

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Opioid system is consists of μ, δ, κ and nociceptin (NOP) receptors and their respective endogenous neuropeptide ligands. In this study we have characterized novel non-mammalian opioid peptides Ile-enkephalin and Phe-enkephalin, and artificial NOP receptor partial agonist hexapeptide Ac-RYYRIR-ol.

Leu- and Met-enkephalin were the first endogenous opioid peptides identified in different mammalian species including the human. Comparative biochemical and bioinformatic evidence indicates that enkephalins are not limited to mammals. Lower vertebrate enkephalins were investigated with in vitro biochemical experiments using rat brain membrane preparations and turned out to be moderate affinity opioids with a definite preference for the δ-opioid receptor sites. Phe-enkephalin from the lungfish displayed low affinities toward the μ- and δ-opioid receptor, while exhibited moderate affinity toward the κ-opioid receptor. In [^{35}S]GTPγS binding studies, Met-enkephalin produced the highest stimulation followed by Leu-enkephalin, Ile-enkephalin from the clawed frog and Phe-enkephalin, was the least efficacious among these endogenous peptides (Bojnik et al. 2009a).

Some N/OFQ sequence unrelated hexapeptides can effectively bind to the NOP receptor and they were used as template for structure activity studies that lead to discovery of the new NOP selective ligands. The pharmacological profile of the novel hexapeptide Ac-RYYRIR-ol was investigated using various in vitro assays including receptor binding and G protein activation in rat brain membranes, mouse vas deferens, rat vas deferens, guinea pig ileum, mouse colon and calcium mobilization. In rat brain membranes Ac-RYYRIR-ol displaced [^{3}H]Ac-RYYRIK-ol (Bojnik et al. 2009b) with high affinity and stimulated [^{35}S]GTPγS binding with high potency. The stimulatory effect of Ac-RYYRIR-ol was antagonized by the selective NOP receptor antagonist UFP-101. In antagonist type experiments Ac-RYYRIR-ol inhibited the stimulatory effects induced by N/OFQ. In the electrically stimulated mouse vas deferens Ac-RYYRIR-ol displayed negligible agonist activity while antagonizing in a competitive manner the inhibitory effects of N/OFQ. In the mouse colon Ac-RYYRIR-ol produced concentration dependent contractile effects with similar potency and maximal effects as N/OFQ. Finally, in the Ca^{2+} mobilization assays Ac-RYYRIR-ol displayed lower potency and maximal effects compared with N/OFQ assays.

In conclusion, two novel, non-mammalian enkephalins were described and compared with those of the well-known Leu- and Met-enkephalin. Among the four structures tested, the 'mammalian type' Met-enkephalin exhibited the highest affinities in receptor binding assays and produced the most efficacious G-protein stimulation in brain membranes, while the newly identified 'lower vertebrate type' Ile- and Phe-enkephalins were found to be less effective. On the other hand, novel NOP receptor selective hexapeptide Ac-RYYRIR-ol has been shown to have fine selectivity, high potency, furthermore agonist and antagonist effects toward the NOP receptors were measured in various assays. This is likely due to its partial agonist pharmacological activity.

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Genetic modification of carotene producing Zygomycetes

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Carotenoids are terpenoid-type chemical compounds. These yellow to orange-red natural pigments are used in the food, pharmaceutical and cosmetic industry and as feed colour additives. Recently, they are attracting an increasing attention, due to their beneficial effects on health. In Zygomycetes fungi, β-carotene is the predominant carotenoid. Traditionally three species: *Blakeslea trispora*, *Phycomyces blakesleeanus* and *Mucor circinelloides* have been involved in the study of the carotene biosynthesis.

Mucor circinelloides has several characteristics advantageous for molecular genetic studies. For example, well functioning methods are available for the genetic transformation of this fungus based on autonomously replicating plasmids (Papp et al. 2008). However, integrative transformation methods are not well established and the fate of the transforming DNA has not yet been analyzed.

The aims of our work were (1) to investigate and compare the effect of overexpression of different isoprene biosynthesis genes for the carotene production; (2) to produce oxygenated β-carotene derivatives by heterologous expression of the *crtW* gene (encoding β-carotene ketolase) of the marine *Agrobacterium aurantiacum*; (3) to integrate the *crtW* gene into the *Mucor* genome by different methods; (4) and to reveal the carotenoid spectra and to characterize the carotenoid production of some Zygomycetes in order to determine new producer strains potentially applicable in further analysis.

Transformation of fungal protoplasts was carried out by the polyethylene glycol-mediated method. Three different isoprenoid genes were involved in the study. Expression vectors that contained one of these genes driven either by their own promoter or by the regulator

sequences of the *Mucor* glyceraldehyde-3-phosphate dehydrogenase 1 gene (*gpd1*), were constructed. The *Mucor leuA* or *pyrG* genes were used as selection markers; they complement the leucine or uracil auxotrophy of the recipient *M. circinelloides* strain, respectively. Vectors were introduced alone ore in co-transformations to combine the isoprenoid genes. All transformants proved to be stable under selective conditions and some of them under non-selective conditions as well. Transformants were analyzed with hybridization and PCR techniques. Real-time PCR analysis revealed a relatively high copy number of the plasmids in the transformants and an unequal proportion of them in the co-transformants. Higher expression of the genes was also verified. The carotene production was analyzed by spectrofotometric, TLC and HPLC methods.

It has been found that *M. circinelloides* has β-carotene hydroxylase activity, therefore introducing the *crtW* gene may result in the production of several types of oxygenated β-carotene derivatives. Transformation with vector, containing the *crtW* gene under the control of *gpd1* promoter, was carried out (Papp et al. 2006) and co-transformations with the isoprene genes were also done. Changes in the carotenoid production due to expression of the *crtW* gene have been proven.

Integration the *crtW* gene into the *Mucor* genome was achieved by three different methods: homologous recombination with double crossing over, *Agrobacterium tumefaciens*-mediated transformation and restriction enzyme-mediated integration. The integration had been proven and analysed in several transformants by PCR, inverse-PCR, real-time PCR and hybridization techniques.

Carotene content of twenty one Zygomycetes strains was also analyzed. Some of them produced the same or higher amount of carotenoids than the wild type *M. circinelloides* or *B. trispora* strains. These strains were analyzed under different conditions, e.g. temperature, light and carbon source. For some of these strains, we started the development of new transformation systems that allows the direct selection of the transformants.

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Increased genetic stability of a rationally designed reduced-genome *Escherichia coli*

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In an attempt to engineer a simplified, core-genome *Escherichia coli*, we have reduced the wild-type K-12 MG1655 genome by making surgically precise scarless deletions (Pósfai et al. 2006). Genome reduction was achieved without compromising the basic metabolic circuits of the cells. The new strains, with genomes up to 22% smaller, were designed by bioinformatic comparative genomics of four *E. coli* strains to identify non-essential genes and recombinogenic, mobile or cryptic virulence sequences, as well as genes with unknown functions for elimination.

These so-called multi-deletion strains (MDS) have several attractive properties which can make them useful in a wide variety of biotechnological applications. One of the most important of these properties is the increased genetic stability of these strains which includes an increase in both genomic and plasmid stability. This work focuses on the quantification of these different aspects of genetic stability. This was done using novel methods we developed for calculating mutation rates (Fehér et al. 2006) as well as rates of recombination within the cells.

Removal of all mobile genetic elements from the *E. coli* genome resulted in a lower mutation rate because of the lack of insertion events. In addition, the genes of three so-called error-prone DNA polymerases (*polB*, *dinB* and *umuDC*) were deleted resulting in a lower point-mutation rate. The resulting strain has a mutation rate that is close to one order of magnitude lower than the wild-type.

In addition to the increased fidelity of replication, lentiviral expression vectors harbored within different MDS strains proved to be more stable than in other commonly used cloning strains (Chakiath and Esposito 2007). By developing a plasmid-based system to measure recombination rates, we were able to quantify this improved stability. The most stable of our strains has a recombination rate that is over five times lower than the wild-type.

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Analysis of a structural motif in the membrane-associated [NiFe] hydrogenases

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Hydrogenases, catalyzing the following reaction: $2H^+ + 2e \Leftrightarrow H_2$, are harbored by numerous microorganisms. The cells dispose excess electrons through hydrogen production accomplished by hydrogenases, while consumption of the molecular hydrogen mostly provides electron source for various energy conserving processes, such as respiration. Sometimes, hydrogen can be the sole energy source for the cell growth. Hydrogenases are distinguished according to the metal content in their active center: they are classified as [NiFe], [FeFe] and [Fe] enzymes (Vignais et al. 2004). Minimally, a [NiFe] hydrogenase is composed of a large and a small subunit and they can be associated to the membrane or localized in the cytoplasm. The large subunit contains a binuclear metallocenter, while the small subunit hosting the Fe-S clusters, which conduct the electrons between the H_2-activating center and the surface of the protein.

Our model organism, the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina* BBS contains at least four active [NiFe] hydrogenases. The HynSL and the HupSL enzymes are attached to the cell membrane, while Hox1YH and Hox2YH are apparently localized in the cytoplasm (Kovacs et al. 2005).

There are several conserved motifs in the sequence of the hydrogenases, which are characteristic for these enzymes. These motifs have very important role for example in coordination of the metals of the active centre, in electron transfer, in interaction with other proteins or in translocation of the fully folded protein (Vignais et al. 2007).

We have noticed a highly conserved histidine-rich region with unknown function in the large subunit of [NiFe] hydrogenases. The HxHxxHxxHxH sequence occurs in the large subunit of all membrane-bound hydrogenases, but only two of these conserved histidines are present in the soluble hydrogenases.

In order to identify the function of this motif, mutant strains were made by site-directed point mutagenesis and their biochemical properties were characterized. The *in vivo* and *in vitro* activity measurements showed that the activity was influenced dramatically only in one of the mutants due to the replacement of the His residue with Ala. Nevertheless, this enzyme still remained in the membrane.

Western hybridization experiments were applied to investigate the proteolytic stability of the enzymes. It was found that the strongly reduced activity of the mutant hydrogenase could not be derived from the destabilization of the protein.

The oxygen sensitivity of the single amino acid mutant and the wild type protein was also compared for explaining the background of significant *in vitro* and *in vivo* activity loss in the mutant strain. The crude extracts from the wild type and mutant cells obtained in anaerobe box and on air was used for *in vitro* spectroscopic measurements, but the ratio of the activities of the wild type and the mutant protein was nearly the same independently on the environment of the cell destruction.

For further biochemical and biophysical investigations, large amount of enzymes have to be purified. First, a HynS-Strep-II Tagged fusion protein was constructed to purify the non-mutant HynSL enzyme by affinity chromatography. However the amount of the purified protein was not enough for further spectroscopic experiments and the specific activity of the Strep-II fused hydrogenase was lower as compared to the natural enzyme. We continued with the standard biochemical techniques and used fast protein liquid chromatography (FPLC) for purification of the wild type and mutant protein in large scale. These experiments are in progress and the first results are very auspicious.

For getting a complete picture about the structure-function relationship in HynSL hydrogenase, other conserved residues are being investigated, as well. From biotechnological point of view thermostability and oxygen tolerance of the hydrogenases are two crucial properties. In order to improve these properties of the enzymes, identification and functional characterization of candidate sequences potentially conferring these beneficial properties to the enzymes are to be done.

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Mechanistic insights into the role of translesion synthesis and its effect on genome stability

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REV1 is a Y-family DNA polymerase. REV1 proteins contain a BRCT domain, which is important in protein-protein interactions. A suggested role for REV1 protein is as a scaffold that recruits DNA polymerases involved in translesion synthesis (TLS) of damaged DNA.

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To elucidate the mechanism by which REV1 promotes DNA damage bypass, we have analyzed the progression of replication on ultraviolet light-damaged DNA in mouse embryonic fibroblasts that contain a defined deletion in the N-terminal BRCT domain of REV1, or that are deficient for REV1.

DNA fiber labeling method that has been previously described has been adapted in which two modified nucleotides IdU and BrdU were used to label newly replicated DNA. Incorporated IdU and BrdU were detected by fluorescent immunolabeling and the progression of replication fork was monitored. To examine the effect of UV damage to DNA on replication fork progression, cells were treated with either 20J/m^2 or 40J/m^2 UV dose at the end of first labeling period (IdU) and before second labeling (BrdU). Fork rates were calculated for each labeling period and the ratio of IdU to BrdU were analyzed. Under normal replication conditions ratio of IdU to BrdU is approximately 1. However an increase in this ratio directly corresponds to the rate of fork stalling during second labeling as a result of UV damage to DNA.

To investigate the role of REV1 BRCT and REV1 in replication fork progression, DNA fiber spreads were prepared, labeled forks were measured and compared to that of wild type cell line. In wild type cells with no UV treatment the average ratio of IdU to BrdU was 1.13 and REV1 BRCT and REV1 mutant cells showed an average ratio of 1.13 and 1.16 respectively. There was no significant difference in the rate of fork progression in any of the mutant cell lines as compared to the wild type line. Therefore, these genes are dispensable for the normal growth and viability of the cell.

The frequency of fork stalling in REV1 BRCT and REV1 mutant cell lines after 20J/m^2 or 40J/m^2 UV dose was measured. Both REV1 BRCT and REV1 mutant cells showed a significant increase in the ratio of IdU to BrdU in response to 20J/m^2 UV treatment and the ratios increased further at 40J/m^2 UV. In wild type cells with 20J/m^2 and 40J/m^2 UV treatment the average ratio of IdU to BrdU increased from 1.13 to 1.92 and 3.24. REV1 BRCT and REV1 mutant cells showed an increase in the ratio from1.13 to 4.14 and 4.99, 1.16 to 3.43 and 4.5 respectively. Furthermore, the exogenous expression of mREV1 in REV1 BRCT and REV1 mutant cell lines restored wild type phenotypes.

These results provide an evidence for the role of BRCT domain of REV1 in response to DNA damage and that REV1 plays a central role in replication fork progression of UV-damaged DNA.

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Uptake and degradation of xenobiotic in *Sphingomonas subarctica* SA1

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Sulfanilic acid is a typical representative of sulfonated aromatic amines widely used and manufactured as an important intermediate in the production of azo dyes, plant protectives and pharmaceuticals. *Sphingomonas subarctica* SA1, a Gram-negative aerob bacterium is able to utilize sulfanilic acid as the only carbon, nitrogen, and sulfur source (Perei et al. 2001). In addition to sulfanilic acid, *Sphingomonas subarctica* SA1 could degrade six other aromatic compounds, such as sulfocatechol, protocatechol, para-amino benzoic acid, 3,5-dihydroxybenzoic acid and oil in soils.

Comparison of the protein patterns of cells grown on various substrates revealed that the strain used alternative metabolic pathways for biodegradation of these compounds. However, similar patterns were observed in the case of cells grown on sulfanilic acid and sulfocatechol. Therefore sulfocatechol is supposed to be formed, as first intermediate in the catabolism of sulfanilic acid. Unfortunately, sulfanilic acid could be converted by intact cells only but not by disrupted cells, thus the characterization of this reaction step was difficult. Nevertheless, sulfocatechol is further oxidized by a ring cleaving dioxygenase, named as sulfocatechol dioxygenase. This enzyme was partially purified and identified by mass spectrometry (Magony et al. 2007). A genomic locus harbouring the genes of sulfocatechol dioxygenase (*scaEF*) was also identified and upstream from these genes, few other *orfs* coding for proteins similar to muconate cycloisomerases (ScaA), lactone hydrolases (ScaB), maleylacetate reductases (ScaC) and an oxidase (ScaD) were recognized. These enzymes were actively overexpressed in *E. coli* and the sulfocatechol degradation pathway was reconstituted by the recombinant proteins.

The first step of sulfanilic acid degradation is not fully understood. The enzymes probably converting sulfanilic acid to sulfocatechol were very sensitive to cell disruption indicating that they were somehow related to the membrane. Proteomics approach was applied to identify of the enzymes catalyzing the sulfanilic acid conversion. Bands sepcifically appearing upon substrate induction in the membrane and soluble fractions were cut out and sequenced *de novo* by mass spectrometry.

The analysis of the proteomic data of the soluble fraction led to the identification of another gene set in the genome. In this locus, two genes likely coding for proteins involved in the oxidative deamination of sulfanilic were predicted.

Three specifically appearing membrane proteins were found in the membrane fractions of cells grown on sulfanilic acid. The aminotransferase is probably one component of the sulfanilic acid converting enzymes catalyzing the deamination of sulfanilic acid. It was shown to be poorly membrane associated, since it was also found in the soluble fractions. The second protein contained motifs of ATP-binding casettes indicating the energy-dependence of sulfanilic acid uptake. The third protein is a hypothetical TonB-dependent protein, which might play a role in many types of transport including iron uptake. The expression of the TonB-dependent protein is upregulated specifically by xenobiotics/aromatics and iron. Since, two enzymes of the degradation pathway are known to contain iron in their active center, it is plausible to assume, that the TonB dependent protein is involved in the iron transport to feed the extra iron demand of the enzymes taking part in the biodegradation.

From our data it is assumed that the uptake and conversion of sulfanilic acid is linked to a membrane protein complex and this association can function as a self-defending mechanism for the cell against the cytoplasmic occurrence of the toxic substrates. Furthermore, a potential link between the xenobiotics degradation and iron transport is suggested.

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Role of salicylic acid pretreatment on the photosynthetic performance and the generation of reactive oxigen species and nitric oxide in tomato plants (*Solanum lycopersicum* Mill. L. cvar. Rio Fuego) under salt stress: acclimatization or programmed cell death

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Salicylic acid (SA) has long been known as a signal molecule in the induction of defense mechanisms in plants (Raskin 1992) and it was shown to improve the acclimation to different abiotic stress factors, including high salinity (Szepesi et al. 2009). SA increased reactive oxygen species (ROS) production and therefore oxidative stress (Knörzer et al. 1999). SA was also shown to influence a number of physiological processes (Raskin 1992) e. g. inhibited the activity of Rubisco and thus the photosynthetic activity (Vernooij et al. 1994).

The aim of my work was to reveal, how ROS production (O^-; H_2O_2) was modified by different concentrations of SA, how the SA-treated cells could acclimate to oxidative stress or why other tissues became committed to programmed cell death (PCD).

On the basis of the inhibity effect of SA on stomatal conductance and photosynthetic performance, which has been documented in several papers, it was presumed, that the ROS produced after SA pretreatment may be derived from an inhibited photosynthetic electron transport. This may also reduce the plants capacity to synthesise compatible osmolytes, such as sugars, during pretreatment or salt stress.

That is why we measured the changes in photosynthetic activity (chl*a* fluorescence induction parameters, CO_2 fixation rate as function of PAR or Ci and stomatal conductance) during pretreatment.

Tomato plants were grown hydroponically in the presence of different SA (10^{-3} M, 10^{-4} M, 10^{-7} M). Seven-week-old plants were exposed to 100 mM NaCl for a week.

Short-term pretreatment of plants with 10^{-3} M SA resulted in a permanent decrease in the stomatal conductivity and the CO_2 fixation rate compared to the control and also decreased the viability of plants. In contrast, after a transient decline photosynthetic parameters of plants grown in 10^{-7} and 10^{-4} M SA were not significantly different from the untreated control at the end of the pretreatment period. Salt stress also inhibited the photosynthetic activity, which was significantly alleviated by 10^{-4} M SA. The improved photosynthetic performance and the accumulation of soluble sugars as compatible osmolytes resulted in a partial osmotic adjustment and contributed to successful acclimation to high salinity in 10^{-4} M SA pretreated plants.

The accumulation of putrescine in the leaves and those of spermidine and spermine in the roots are adaptive feature of some halophyte species. We found similar changes in the polyamine spectrum of plants grown in 10^{-4} M SA at the end of pre-treatment period. Moreover these tissues produced less ethylene, a PCD inducing plant hormone, which coincided with higher viability of root apical cells.

As it was expected a significant accumulation of H_2O_2 occurred in the leaves and roots of plants exposed to 10^{-4}-10^{-7} M SA, but after three weeks the differences disappeared in the root tissues and remained in the leaves. We prepared mesophyll protoplasts as model system to investigate the effects of the compounds that accumulated in plants during pre-treatments on ROS production and to compare the results with intact plants.

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The role of Drosophila formin dDAAM in axon growth

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In the developing nervous system, the growth cones have an essential role in guiding the axons to their targets. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. The peripheral F-actin is organized into long bundled actin filaments in the finger-like filopodia and diffuse networks of shorter actin filaments contained in the veil-like lamellipodia. Previous work identified many immediate regulators of F-actin dynamics in growth cones, and for some of these, it has been demonstrated that they act downstream of signaling pathways involved in axonal growth regulation. Key regulators of actin dynamics are the so called nucleation factors, such as the Arp2/3 complex and formins, which use different mechanisms to seed new actin filaments (Pak CW et al. 2008).

The formin proteins are involved in actin polymerization and growth by associating with the fast-growing end (barbed end) of actin filaments. Formins contain two homology domains the FH1, and the FH2. The actin nucleation-promoting activity of formins has been localized to the FH1-FH2 domains. The FH1 domain is a proline-rich region, that can bind the G-actin monomer carrying profilin protein. The FH2 domain is necessary and sufficient to nucleate actin in vitro (Goode BL and Eck MJ 2007).

The principal field of research in our group is the functional analysis of the single *Drosophila DAAM* ortholog. We revealed that *dDAAM* is transcribed pan-neurally from stage 11 of embryogenesis in the area of central nervous system (CNS). The immunostaining experiments demonstrated that dDAAM protein is highly enriched in neurites, where it shows a strong colocalization with actin. Therefore we wanted to investigate if this protein plays a role in neurite growth.

To examine the function of *dDAAM* in the CNS, we first carried out a loss of function (LOF) analysis by examining mutant embryonic ventral nerve cord and primary neuronal cultures. The mutant embryos showed strong CNS phenotypes, displayed frequent breaks in the connectives and commissures. The *dDAAM* mutant neurons were able to develop axons of similar length as their wild-type counterparts, but the filopodia of mutant neurons were reduced in number and in length. Thus, the LOF experiments suggest that *dDAAM* regulates filopodium formation in neuronal growth cones.

Next we examined the effect of the constitutively active form of dDAAM on the embryonic CNS. We detected that the overexpression of this form in the embryonic CNS results in severe fasciculation defects and embryonic lethality. To collect additional evidences that activated dDAAM has the potential to increase neurite number, we examined embryonic nerve cord cultures overexpressing this form. The ventral nerve cords expressing activated dDAAM exhibited a much denser neuritic meshwork and growed more extended axons than their wild type counterparts. In cultured primary neurons, the overexpression of activated protein increased the number of filopodia. These observations suggest that dDAAM plays a major role in the regulation of axonal growth, presumably by promoting actin assembly in the growth cone (Matusek et al. 2008).

In addition, we detected strong dDAAM expression in adult brain as well. In LOF analysis we found several axonal projection defects indicating a role in the formation of the adult neuropil.

To determine proteins that may act together with dDAAM in the regulation of axonal growth, we carried out a genetic interaction analysis. We demonstrated that dDAAM shows an interaction with *Ena and profilin*. Moreover, we identified Rac as the most likely activator of dDAAM in the developing nervous system. (Matusek et al. 2008).

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Biohydrogen production using the cellulose containing plant biomass

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There are many serious gobal problems caused by mankind in the last dacades. Among these, two have oustanding importance: a) the enviromentally friendly, biological degradation of the large amounts of organic waste poduced by the industrial sector; b) as well as the reinforcement of the energy supply by renewable energy source and the substitution of the current energy carriers by environmentally sound fuels. Hydrogen is considered as the best candidate for the future energy carriers, since just pure water is formed during its oxidation. There are biological tools for producing hydrogen and hydrogen evolving photosynthetic or fermentative microbes are primarily involved in these processes. In the dark fermantative processes, usually biopolymers of agricultural origin used to be the substrate, which have to be first converted to simpler monomers being fed to the hydrogen producing bacteria. Strains capable to convert and utilize complex biopolymers are of extrme importance. One of the best candidates is the Gram positive, hyperthermophilic, anaerobic *Caldicellulosiruptor saccharolyticus.* (Bagi et al. 2007) Its biotechnological importance is that it is capable to degrade cellulose-based biomasses, such as paper, or energy plants (which are found in the large quantities) and has hydrogenases for removal of excess electrons formed during the fermentative metabolism. The genome of this bacterium is available and the strain was shown to possess not only numerous glycosidases required for the hydrolysis of different kinds of polysaccharides, but also hydrogenase enzymes responsible for hydrogen production.

In our group an enviromentally friendly biological method have alredy been developed by wich we waste of animal origin could be transformed to hydrogen. (Bálint et al. 2005.)

In my work I aim at adapting this process to cellulose-based waste of plant origin. Morover,on the basis of the known genome of this organism, I intend to create a genetically modified strain which is capable of degrading the avaible biomass (currently filter paper) more efficiently and thereby producing significantly higher amounts of hydrogen.

According to our aims, I studied the hydrolysis of untreated filter paper in batch fermentation conditions in the presence of six various kinds of sugar using minimal media containing no other carbon sources. In paralel, I monitored the hydrogen evolution from sugars and filter paper alone and various combinations.

My results clearly showed, that *C. saccharolitycus* was able to degrade the untreated filter paper and to produce abundance hydrogen from this material in the presence of minimal amount of sugar. From the sugar specificity, it is likely that these sugars are basically necessary for the induction of cellulase enzymes., thus paper decomposition can be promoted by sugars.

For better understanding the molecular background of the events and for further improvement of the process genetically modified strains should be constructed. However, no protocol is available for gene transfer into *C. saccharolyticus*. In order to introduce foreign DNS into the cells, a functional genetic system has to be developed. As a first step, the efficient plating technique was ameliorated and numerous suitable vectors (replicative and non replicative) were collected and constructed to achieve sustainable vector replication or to modify the given part of the genome. Furthermore the development of an efficient method for introduction of foreign DNA is also requisite.

My future plans are the optimization of the paper decomposition conditions, getting deeper insight into the the molecular mechanism of the paper/sugar metabolism → hydrogen conversion.

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Construction and characterization of synthetic genetic oscillator in yeast

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The design of gene expression systems with both spatial and temporal regulation has been an area of intense scientific interest during the last ten years. Our aim is to create a synthetic genetic circuit in yeast based on transcriptional – translational feedback loops mimicking the structure (input, oscillator, output) and function (self-sustaining oscillation and resetting) of eukaryotic circadian clocks. The circuit will serve as an ideal test system for mathematical modeling describing oscillatory mechanisms (eg. circadian clocks), since all components are known, well characterized and can be easily modified/adjusted in order to test predictions from the model. The main requirements for such a system are: a genetic network with well defined components, which do not interfere with the physiology of yeast; option to set/modify the expression level/turn-over rates of components, proper input/resetting mechanism and easily measurable output (in vivo, real-time).

First we created and tested components for the input pathway of the oscillator. In the circadian systems of eukaryotes, external signals (e.g. light and/or temperature) reach the oscillating genetic network through the input pathway and cause an acute change in the expression

or stability of one or more oscillator components, which consequently resets the phase of the oscillation. In our system, light act as a resetting signal via an artificial light switch. The switch is based on the light-dependent interaction between the plant photoreceptor phytochrome A (PHYA) and its specific interacting protein FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) [Hiltbrunner, 2005]. The activator and the DNA-binding domain of the GAL4 transcription factor are fused to FHY1 and PHYA, respectively, so transcription from GAL4-dependent promoters is activated only by the physical interaction between PHYA and FHY1 in mutant yeast cells lacking the endogenous GAL4 protein. Red light converts PHYA to its active form, which interacts with FHY1; however, far-red light diminishes the interaction, because it converts PHYA to its inactive conformer. To test the function of the light switch we measured expression of the GAL1 promoter driven luciferase reporter gene (GAL1:LUC+). We showed that luciferase activity is tightly controlled by red or far-red light pulses indicating the proper function of the light switch.

The core components of the oscillator (termed "Yeascillator") are represented by two artificial genes whose gene products can mutually regulate transcription of each other. Expression of the positive protein [Gari, 1997] is driven by a modular promoter, which contains cis-elements for copper induction and for binding of the negative component protein. The basal activity of this promoter is controlled by copper in the media. The positive protein is a fusion between the tetracycline-responsive transactivator (tTA) and the YFP proteins. tTA-YFP is able to bind to a specific cis-element (*tetO*) built in the promoter of the second gene encoding the negative protein. Binding of tTA-YFP can be controlled by doxycycline and results in the activation transcription. The negative protein consists of the DNA-binding domain of the bacterial LexA protein, the yeast transcriptional repressor SSN6 and the CFP proteins. LexA-CFP-SSN6 binds to the modular promoter of the first gene via specific LexA binding sites and represses transcription. The different fluorescent protein tags (YFP and CFP) allow simultaneous detection and quantification of the positive and negative protein components. The output of the oscillator is represented LUC+ reporter gene controlled by a promoter, which responds to the positive component only. Our preliminary results indicate that the two genes can regulate each other as expected, but detection of oscillation will require more optimizations.

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Regulation of single spike initiated feed-forward networks through 5-HT-2 receptors in the human and rat cerebral cortex

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The performance of the human cerebral cortex is unparalleled by the nervous system of other species and this is presumably supported by refined, but largely unknown features of the human microcircuit. We have shown that single action potentials in pyramidal cells can trigger reliable and stereotyped series of multiple postsynaptic potentials in simultaneously recorded pyramidal cells and interneurons in the human cerebral cortex. These polysynaptic event series are composed of alternating excitatory and inhibitory postsynaptic potentials lasting up to tens of milliseconds (Molnar, Olah et al. 2008).

We tested how these complex network events could be affected by the endogenous neurotransmitter serotonin known to be involved in several physiological processes, and implicated in many psychiatric disorders (Jones and Blackburn 2002). We recorded from pairs, triplets and quadruplets of neurons in slices of human association cortices looking for mono- and polysynaptic connections. Nanomolar concentrations of serotonin reversibly supressed single pyramidal spike activated di- and polysynaptic events, and this effect could be mimicked by the 5-HT-2 receptor agonist alpha-methylserotonin. Similarly, alpha-methylserotonin was effective in eliminating axo-axonic cell triggered polysynaptic but not disynaptic events.

We then investigated the effect of serotonin on monosynaptic unitary connections between various types of layer 2/3 neurons. We found that serotonin and alpha-methylserotonin decreased the amplitude of EPSPs between pyramidal cells and from pyramidal to various types of interneurons including fast-spiking basket and axo-axonic cells but little or did not change the amplitude of IPSPs from fast-spiking to pyramidal neurons.

To examine the mechanism by which serotonin might modulate excitatory transmission, we analysed the percentage of failures to evoke an EPSP and the coefficient of variation of unitary EPSP amplitudes with and without serotonin and alpha-methylserotonin. Both serotonin and alpha-methylserotonin increased the failure rate and the coefficient of variation suggesting a presynaptic site of modulation of serotonin in the glutamatergic synaptic transmission.

Finally, we found that therapeutic concentrations of the serotonin reuptake inhibitor fluoxetine, a widely prescribed medication for treatment of depression, could enhance the effect of serotonin on excitatory synaptic transmission.

In conclusion, activation of 5-HT2 receptors can eliminate pyramidal cell activated feed-forward network events presumably via down-regulation of glutamate release probability in pyramidal axon terminals.

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Identification of potential mycotoxin producing *Fusarium* species in Hungarian wheat grain samples

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Fusarium Head Blight (FHB) is a disease complex of cereals, in which several fungal species may cause symptoms. The species found as the major cause of head blight of wheat are *F. graminearum* and *F. culmorum*. Less frequently isolated species are *F. acuminatum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides*. FHB can significantly reduce grain yield and quality.

Fusarium species are known as mycotoxin producers. The most predominant mycotoxins found in small-grain cereals are 8-ketotrichothecenes such as deoxynivalenol (DON) and nivalenol (NIV) and their acetylated derivatives including 3-acetyldeoxynivalenol (3-ADON) and 15-ADON, as well as an oestrogenic mycotoxin, zearalenone (Mirocha et al. 1989). A less frequently examined mycotoxin group is of the enniatins (ENs). *F. avenaceum*, *F. poae*, *F. sporotrichioides* and *F. tricinctum* are the main sources of ENs (Nicholson et al. 2004, Ivanova et al. 2006).

Species identification of mycotoxin-producing *Fusarium* species is of high importance in relation to FHB. The polymerase chain reaction (PCR) is a useful technique for the identification and differentiation of *Fusarium* species.

The aim of this study was to apply species-specific PCR-based assay for the identification of *Fusarium* species from Hungarian wheat grains. After processing 75 wheat samples of different geographical origin we isolated 255 *Fusarium* strains. Identification with species-specific PCR primers revealed the following species distribution: *F. acuminatum* 7.5 %, *F. avenaceum* 8.5 %, *F. graminearum* 37.5 %, *F. poae* 30.5 % and *F. sporotrichioides* 16 %. The results were confirmed by morphological identification after culturing the isolates on potato dextrose agar plates.

In addition to the species identification, we also performed PCR reactions to reveal the presence/absence of genes responsible for the production of several toxins (DON, 3-ADON, 15-ADON, NIV and ENs) in the *Fusarium* isolates. *F. graminearum* proved to be the most important fungus responsible for different diseases of small-grain cereals in Hungary. We used diagnostic primer sets, based on the *Tri3* (3-ADON and 15-ADON), *Tri5* (DON) and *Tri7* (NIV) trichothecene genes (Qurta et al. 2006), in multiplex PCR for the detection of *F. graminearum* chemotypes. An additional primer set was used to detect the *esyn1* gene (Kulik et al. 2007) for the detection of potential enniatin-producing *F. avenaceum*, *F. poae* and *F. sporotrichioides* species.

The investigated 96 *F. graminearum* isolates were potential producers of both DON and NIV toxins. The chemotypes were the following: DON 1 isolate, DON-NIV 1 isolate, DON-15-ADON 94 isolates. There was no isolate found containing the gene responsible for 3-ADON production. Our results suggest that strains of *F. graminearum* prevailing in Hungarian wheat-growing regions belong mainly to the DON-15-ADON chemotype.

Each of the examined *F. avenaceum*, *F. poae* and *F. sporotrichioides* isolates were positive for the *esyn1* gene, except two *F. poae* isolates. This is the first data set of enniatin-producing potential of Hungarian *Fusarium* isolates and shows that toxic potential of these strains may be underestimated.

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Phosphatidylglycerol is important in the assembly and function of PSII reaction center

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Phosphatidylglycerol (PG) is a ubiquitous anionic phospholipid in almost all organisms. The structural and functional roles of anionic lipids in photosynthesis have raised scientific interest for a long time. The role of PG in photosynthetic organisms has previously been studied using either biochemical or molecular genetic approaches. The recent identification of genes encoding enzymes required for the biosynthesis of PG in cyanobacteria and eukaryotic plants, and the subsequent generation of mutants defective in the biosynthesis of PG, has provided powerful molecular tools to understand the function of PG in photosynthetic organisms. The role of PG has been extensively studied in two PG-less mutant strain of *Synechocystis sp.* PCC6803: Δ*pgsA* (Hagio 2000) and Δ*cdsA* (Sato 2000). Previously it was demonstrated that PG is required for the formation and function of thylakoid membranes in cyanobacteria and plants (Wada and Murata 2007; Domonkos 2008).

In the present investigation we constructed and characterized a new PG deficient mutant of *Synechocystis sp*. PCC6803. We inactivated the *cdsA* gene in phycobiliproteinless mutant, PAL, which compensates the missing light harvesting complex by high cellular content of PSII (Ajlani 1998). The PAL/Δ*cdsA* mutant provided a unique experimental system for a more detailed study of the role of PG in PSII function/assembly. We analyzed the influence of PG depletion on the fluorescence induction, thermoluminescence, biosynthesis and assembly of PSII protein subunits. The mutant cells grew only in a medium supplemented with PG. Depletion of PG in the cells resulted (i) in an inhibition of cell growth/division, (ii) in a small change in pigment composition, (iii) in the inactivation of oxygen evolution, (iv) in a modification of the fluorescence induction curve that pointed to some damage of Q_B, but not the donor side, (v) in a modification of the TL glow curve to give only shifted Q-band which is an indicator for suppression of electron transfer between Q_A and Q_B, and it does not affect the redox levels of Q_A and S_2. Two-dimensional PAGE showed that in the absence of PG (a) PSII dimer was monomerised, and (b) the CP43 protein was detached from a major part of the PSII core complex. [35S]-methionine labeling confirmed that PG depletion did not block de novo synthesis of PSII proteins. We conclude that PG is required for the binding of CP43 within the PSII core complex (Laczko-Dobos 2008). This is in good agreement with the presence of a PG molecule localized between D1 and CP43 subunits by X-ray crystallographic structure of *Thermosynecococcus elongatus* (Guskov 2009).

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Characterization of catalase genes in *Rhizopus oryzae*

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Zygomycosis is a diverse group of mycotic diseases caused by members of the class Zygomycetes. The main risk factors are diabetic ketoacidosis; cancer and its therapy; solid organ or bone marrow transplantations; prolonged steroid use; neutropaenia; deferoxamine treatment to manage iron overload and burn injuries (Papp et al. 2008). Thermophilic members of the genus *Rhizopus*, especially *R. oryzae*, are considered as the main causative agents of zygomycoses. During the past decades, such infections have emerged in an increasing number due to the widespread use of immunosuppressive therapy, intensive cancer chemotherapeutic regimens and broad-spectrum antimicrobial agents. High mortality rates, difficulties in the diagnosis and resistance to the most widely used antifungal drugs are characteristic features of zygomycoses underlying the importance of this fungal group (Ribes et al. 2000). All these aspects indicate that development of new strategies to prevent and treat these infections is urgently needed.

The aim of our study is identification and analysis of the genetic background of the virulence of opportunistic pathogen Zygomycetes.

Generation of oxidative products by phagocytic cells is known to be one of the important host defence mechanisms directed towards the killing of invading microorganisms (Gallin at al. 1993). Catalases may provide protection against reactive oxygen species produced by neutrophile granulocytes of the human immune system (Chang et al. 1998). Neutropenia is a considerable risk factor of zygomycoses. In this study, catalase encoding genes of *R. oryzae* have been isolated, and their functional analysis has been started.

Four possible catalase genes were found in the *R. oryzae* genome database (*Rhizopus oryzae* Sequencing Project) by similarity searches with known fungal catalases. These genes and their adjacent regions were amplified by PCR from the genomic DNA of *R. oryzae* and cloned into the vector pBluescriptII SK+ (Stratagene). To reveal their function and to investigate their possible role in the pathogenicity, deletion mutants were created in the case of each isolated genes. Four vectors suitable to create deletions in the different genes were constructed; in each vector, the *pyrG* gene of *R. oryzae* encoding orotidine-5'-monophosphate decarboxylase was placed between the 5' and 3' flanking regions of the appropriate catalase genes. To ensure double crossover gene replacement, linear fragments were cut from the plasmids and used to transform protoplasts of a uracil auxotrophic *R. oryzae* strain using the polyethylene glycol-mediated method.

Integration of the transferred DNAs into the host genome and deletion of the appropriate catalase genes was proven by PCR and Southern blot analysis. Catalase activity of the recipient strain and the four mutants constructed were *in vitro* tested. Effect of hydrogen peroxide on the fungal growth was examined on agar plates and in a microtiter plate assay. All four catalase genes proved to be functional. In all types of mutants, deletion of a catalase gene increased markedly the sensitivity of the transformants to hydrogen peroxide. The strain deficient in the gene designated as 16995 was the less susceptible to hydrogen peroxide whereas the strains deficient in the other genes proved to be more sensitive. Further gene expression studies with isolated genes are in progress and we also plan to use the constructed deletion mutants in pathogenicity tests.

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Comprehensive genetic and biochemical examination of the polyubiquitin receptors in *Drosophila melanogaster*

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The ubiquitin-proteasome system is responsible for the polyubiquitilation and selective degradation of damaged, misfolded and short-lived regulatory proteins to ensure the proper homeostasis of the eukaryotic cell. Recognition of polyubiquitinated substrates by the proteasome is a highly regulated process that requires polyubiquitin receptors (p54/Rpn10: proteasome receptor subunit; Dsk2 and Rad23: non-proteasomal receptors). The mechanism of substrate recognition and delivery to the proteasome is well known in single cell eukaryotes (e.g. yeast), but unresolved in Metazoans.

We found that the subunit composition of the regulatory particle (RC) of the *Drosophila* 26S proteasome changes in a developmentally regulated fashion (Lipinszki et. al. submitted manuscript). The concentration of the p54/Rpn10 subunit falls suddenly at the end of embryogenesis, remains low throughout the larval stages, starts to increase again in the late third instar larvae and remains high in pupae and adults. A similar developmentally regulated fluctuation could be observed in the concentrations of the Rad23 and Dsk2 extraproteasomal polyubiquitin receptors. Our *in vitro* experiments revealed that protein extracts of first or second instar larvae can selectively degrade the embryonic p54/Rpn10 subunit of the 26S proteasome and the Dsk2 and Rad23 polyubiquitin receptors; whereas all the other tested proteins remained intact. The above observations and the fact that the gene expressions of the receptors remain constant during the development suggest that a selective protease is activated during the early larval stages. We successfully purified and identified this protease. Moreover, all the three receptors carry an extended intrinsically unstructured segment within the molecule, which can be the hot spot for the regulator protease.

To follow the *in vivo* fate of subunit p54/Rpn10, transgenic *Drosophila melanogaster* lines encoding the N-terminal half (NTH), the C-terminal half (CTH) or the full-length p54/Rpn10 subunit have been established in the inducible Gal4-UAS system. The daughterless-Gal4 driven whole-body expression of the full-length subunit or its NTH did not produce any detectable phenotypic changes and the transgenic products were incorporated into the 26S proteasome. The transgene-encoded CTH was not incorporated into the 26S proteasome, caused third instar larval lethality and it was found to be multiubiquitinated. This modification, however, did not appear to be a degradation signal, the half-life of the CTH is over 48 hours. The accumulation of the CTH disturbed the developmentally regulated changes of the subunit composition of the RP and, interestingly, the emergence of the selective proteolytic activity responsible for the depletion of the polyubiquitin

receptors. The accumulation of CTH also suspended the MG132 insensitive (proteasome inhibitor), but PMSF (serine-type endopeptidase inhibitor) sensitive proteolytic degradation of the p54/Rpn10, Dsk2 and Rad23 during the early larval stages.

Interestingly, CTH carrying three active UIM sequences extra-proteasomally traps the Dsk2 protein, hindering its interaction with the 26S proteasome. Our *in vitro* and *in vivo* studies revealed that in *Drosophila* UIM motifs of p54 can selectively bound the N-terminal UBL (ubiquitin like) domain of Dsk2. We suppose that contrary to the yeast model in which Rpn1 and Rpn2 scaffold subunits of the RC anchor Dsk2, Rad23 and Ddi1, in *Drosophila* the major polyubiquitin receptor Dsk2 (Lipinszki et. al. manuscript in preparation) docs to the C-terminally localized UIMs of the p54. Nevertheless, is has been demonstrated that p54 is a shuttling subunit of the proteasome (Kiss et. al., Szabó et. al.). It is conceivable that under regulation (e.g. ubiquitilation) p54 dissociates from the proteasome, and forms a heteromer with the Dsk2/substrate dimer, which is followed by the reassociation of the whole complex to the proteasome for degradation of the substrate protein.

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Neuroprotection in ischemic adult rat brain

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Transient global ischemia elicits selective, delayed neuronal death. If the ischemia is short, neuronal damage occurs only in vulnerable areas (Pulsinelli et al. 1985). The pyramidal neurons in the hippocampal CA1 region are very vulnerable. Global ischemia impairs memory and learning functions. It is widely accepted that activation of the excitatory amino acid receptors plays an important role in neuronal death in stroke (Choi 1988). It has recently been reported that glutamate-induced excitotoxicity and a cellular calcium overload are among the key factors of cell death in brain ischemia, especially in the gray matter. By definition, excitotoxicity is a result of overexcitation of the glutamate receptors. In turn, neuroprotective strategies have utilized antagonists of the glutamate receptors to prevent excitotoxic neuronal loss.

The neuroprotective effect of L-kynurenine sulfate (KYN) was studied. KYN pretreatment decreased the number of injured pyramidal cells in the CA1 region of the hippocampus in the four-vessel occlusion (4VO)-induced ischemic adult rat brain. KYN post-treatment proved to be much less effective. In parallel with the histology, a protective effect of KYN on the functioning of the CA1 region was observed: long-term potentiation (LTP) was abolished in the 4VO animals, but its level and duration were restored by pretreatment with KYN. It is concluded that the administration of KYN elevates the KYNA concentration in the brain to neuroprotective levels (Sas et al. 2008).

The excess Glu which causes neuronal death via excitotoxicity, is normally controlled by members of a family of Na^+-dependent Glu transporters. By pumping Glu, they guarantee the presence of Glu in brain fluids at levels at which its exerts neither excitotoxic nor unsolicited excitatory effects. Glu transporters located on the brain vasculature may also play an important role in controlling extracellular Glu levels via a brain-to-blood Glu efflux. The scavenging of blood Glu increases the driving force for the brain-to-blood Glu efflux and causes a decrease of the excess Glu present in the brain. (Teichberg et al. 2008)

In the second series of experiments we evaluated the effects of the blood glutamate scavenger oxaloacetate on the impaired LTP observed in the rat 2-vessel occlusion ischemia model. Transient incomplete forebrain ischaemia was produced 3 days before LTP induction. Although the short transient brain ischaemia did not induce histologically identifiable injuries, it resulted in an impaired LTP function in the hippocampal CA1 region without damaging the basal synaptic transmission between the Schaffer collaterals and the pyramidal neurons. This impairment could be fended off in a dose-dependent manner by the i.v. administration of oxaloacetate immediately after the transient hypoperfusion. These results suggest that oxaloacetate-mediated blood and brain glutamate scavenging contributes to the restoration of the LTP after its impairment by brain ischaemia. (Marosi et al. 2009)

Our results suggest that both agents have potential clinical usefulness for the prevention of neuronal loss in stroke conditions.

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Functional characterization of candidate genes in barley: transgenic plants and grown cultivars

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Barley (*Hordeum vulgare L.*) is one of the major and most distributed crops in the world. Currently it is becoming a novel cereal model plant representing a number of small-grain cereal species. While the barley genome is similar to that of other cereals, it is amenable to explorations of molecular genetics through its true diploidy.

The first Agrobacterium-mediated barley transformation reports was published in 1997 by Tingay and co-workers, using the variety called Golden Promise. The method that we established here was developed for this model cultivar and it's based upon protocols by Trifonova et al. (2001) and Kumlehn (IPK, Gatersleben, unpublished).

The selected genes we used for the plant transformations can be divided into the following three subgroups:

1. The regulators of the cell divison cycle: *MsCDKB2;1* (a *Medicago sativa* cyclin dependent kinase which plays a central role in regulation of the cell cycle, in particular in the G2/M phase transition and in mitosis). In previous studies it revealed that the overproduction of the *MsCDKB2;1* resulted in significant changes in agronomically important parameters in transgenic rice (Lendvai et al., unpublished). Other genes of this group, like OsPP2A B" regulatory subunit, OsRBRI2, OsRBRI5 are previously identified interactors of rice retinoblastoma-related protein, OsRBR1. Since cell cycle regulatory functions of the retinoblastoma proteins are primarily modulated by changing their phosphorylation status, *in planta* studies of the OsRBR1 interaction partner, the OsPP2A protien phosphatase B" regulatory subunit is particularly important from this viewpoint.

2. The 'oxidative stress-defense genes'. First transformation from this group of genes were made by the alfalfa aldo-keto reductase, *MsALR.* This enzyme plays important role in detoxification of the reactive aldehydes issued during oxidative stress, and helps the recovery of the plants (Oberschall et al. 2000). In order to accumulate protective enzymes in different subcellular compartments we constructed a vector for chloroplast targeting of protective enzymes using the transit peptide encoding region of the barley Rubisco LSU gene.

3. The genes involved in grain size determination (*GW2*, *GIF1)*. Loss of *GW2* function increased grain width, weight and yield (Song et al. 2007) Antisense approach results increased grain size, even with constitutive expression of gene fragment in transgenic rice. We have identified and cloned the homologous gene from barley, a specific fragment of it was used for the generation of *HvGW2* antisense plants. *GIF1* (*GRAIN INCOMPLETE FILLING 1*) gene that encodes a cell-wall invertase required for carbon partitioning during early grain-filling (Wang et al. 2008). *GIF1* is responsible for grain weight reduction, ectopic expression of the cultivated *GIF1* gene with the 35S or rice Waxy promoter resulted in smaller grains, whereas over-expression of GIF1 driven by its native promoter increased grain production. These findings, suggest that *GIF1* is a potential domestication gene and that such a domestication-selected gene can be used for further crop improvement.

Establishing a reliable barley transformation technology is very important for the functional characterization of candidate genes and and the produced transgenic lines are subject for further studies.

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Molecular basis of the blood-brain barrier function

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One of the most important functions of the mammalian blood-brain barrier (BBB) is to restrict the free movement of different substances between blood and neural tissue, and it plays a key role in the homeostasis of the central nervous system. The principal components of the BBB are the cerebral endothelial cells that form a continuous monolayer and are interconnected with tight junctions and adherens

junctions. The tight junctions are composed of transmembrane proteins (occludins, claudins, junctional adhesion molecules) connected to junctional plaque proteins (*i.e.* ZO-1). The transmembrane proteins of the adherens junctions are the cadherins linked through catenins (alpha, beta, gamma) to the cytoskeleton.

The blood-brain barrier is involved in a large variety of pathological processes. Little is known about the effects of nicotine exposure on BBB function. We investigated the changes affecting the tight and adherens junction proteins by cigarette smoke components, especially nicotine and polyaromatic hydrocarbons (PAHs). 24h treatment of cerebral endothelial cells with relatively high concentration nicotine led to a decrease in occludin, cadherin and ZO-1 expression. Similar but less pronounced effects were observed after 24 h treatment with phenanthrene. Results of the immunofluorescent analysis confirmed western blot data. We also performed two dimensional electrophoresis in order to explore the cellular proteins responsive to nicotine and PAHs in brain endothelial cells. We observed different responses of the cells to both nicotine and phenanthrene treatment resulting in altered expression of shock induced proteins, metabolic enzymes, signaling molecules. This confirms the cerebral endothelium as being a target to cigarette smoke components (Hutamekalin et al. 2008).

From clinical point of view, because of the relative impermeability of the barrier many drugs are unable to reach the CNS in therapeutically relevant concentration, making the BBB one of the major impediments in the treatment of CNS disorders. A number of strategies have been developed to circumvent this problem. One of the successfully used methods to deliver drugs – especially antitumoral agents – to the CNS is the osmotic opening of the BBB using mannitol. This causes a rapid opening (within minutes) of the BBB which is reversible.

We investigated the effect of mannitol treatment on brain endothelial cells and found that mannitol induced a rapid, concentration dependent, and reversible tyrosine phosphorylation of a broad range of proteins between 50 and 190 kDa. One of the targets of tyrosine phosphorylation turned out to be the adherens junction protein beta-catenin and this phosphorylation was Src-kinase dependent (Farkas et al. 2005).

Beside beta-catenin and Src kinase, we aimed to find new signaling pathways activated by hypertonicity in cerebral endothelial cells and identified the receptor tyrosine kinase Axl to become tyrosine phosphorylated in response to hyperosmotic mannitol. Besides activation, Axl was also cleaved in response to osmotic stress. Specific knockdown of Axl increased the rate of apoptosis in hyperosmotic mannitol-treated cells; therefore, we assume that activation of Axl may be a protective mechanism against hypertonicity-induced apoptosis. Our results identify Axl as an important element of osmotic stress-induced signalling. (Wilhelm et al. 2008).

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Investigation of the maturation of NiFe hydrogenases in *Thiocapsa roseopersicina*

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Our model organism, *Thiocapsa roseopersicina* BBS is an anaerobic, phototrophic purple sulfur bacterium. There are at least two membrane-bound (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe] hydrogenases in the cells. A typical [NiFe] hydrogenase is composed of a large and a small subunit. The large subunit harbors a specific NiFe catalytic metallocenter associated with CO and CN ligands (Volbeda et al. 1995.) The maturation of these complex enzymes require numerous accessory proteins. Most of these auxiliary genes were found using transposon mutagenesis, one of them was the *hupK* gene. (Maróti et al. 2003.) The product of this gene, the HupK protein is present only in organisms containing at least one membrane-bound [NiFe] hydrogenase enzyme.

The role of HupK is not known yet. In order to investigate the role of this protein, Δ*hupK* mutant strains were created, then the hydrogenase activities of the wild type and the mutant strains were compared. The results clearly showed that HupK protein is important for the formation of the functionally active membrane-bound hydrogenases, but not for the biosynthesis of the soluble enzyme. (Maróti et al. 2003.)

More detailed information can be obtained from biochemical experiments. Special expression vector was used to produce active, tagged HupK protein in homologous host. The tagged HupK protein was purified under mild conditions to retain all protein-protein interactions and the copurified proteins were analyzed by mass spectrometry. From cells grown under standard conditions, only one protein partner, namely the GroEL chaperonin could be fished out. The specific role of GroEL in the hydrogenase maturation is not likely, therefore alternative growth conditions were used to find the specific partners. Nickel starvation of the cells is supposed to result in the accumulation of the intermediates of the posttranslational process. Therefore, the tagged HupK protein was purified from such cells, however only one co-purifying band was observed: the PuhA protein, which is the H subunit of the photosynthetic reaction centre.

Metal content determination of the purified HupK protein from homologous host was performed. The HupK protein was shown to contain nickel atom in 1:2 molar ratio, and no Fe atom was detected in the sample. In order to determine, which amino acids assist in binding the nickel atom, the sequences of the HupK and the large subunits of the hydrogenases were compared. Conserved regions could be recognized at the N- and C-termini, while the middle part of the proteins was variable. The alignment uncovered two conserved cysteine residues as candidates for coordination of the metal. One of them is in the R-X-F-X-X-C motif at the amino terminus, other one is in the D-P-C-X-X-F motif at the carboxyl terminus. In order to examine the putative role of these residues, site-directed mutagenesis were performed and the effects of the mutations were monitored via the hydrogenase activties of the membrane-associated hydrogenases. A mutant carrying alanine instead of Cys378 had only 65% activity of the wild-type strain. However, the replacement of the Cys54 by alanine led to a considerable reduction in the hydrogenase activity (to 25% of the wild type level). Ni content investigation of the Cys54Ala mutant HupK protein revealed that it contains the same amount of Ni atom like the wild-type protein.

The R-X-C-X-X-C and the D-P-C-X-X-C sequences in the large subunits of NiFe hydrogenases have been shown to be essential for their activity and the cysteine residues have been proposed to form a coordination sphere surrounding the NiFe center (Przybyla et al. 1992, Volbeda et al.,1995.). In the HupK protein, phenylalanines subtitute the first and the last cysteins. In order to confer the motifs of the hydrogenas large subunit on HupK, the two phenylalanines were replaced by cysteines. The effect of these mutations on the biosynthesis of the membrane-bound hydrogenase is being investigated.

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Anthropological Analysis of the Medieval Cemetery of 'Szeged-Vár'

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The environs of Szeged was a populated area from the primitive age; archeological finds prove there were Roman inhabitants at the time of Roman Empire, and later habitation during the great migrations of Huns, Gepids and Avars. This area was important because this is where Maros runs into the Tisza River, the Maros River being an excellent and cheap transport possibility of salt from Transylvania.

The first mention of the Medieval town is from 1246. As a result of the Turkish occupation of Hungary limited data is available, making the archeological excavation of Szeged-Vár very important.

The Medieval cemetery of Szeged-Vár was used from the Hungarian conquest to 1543, and from 1686 until 1713. The excavations have been going on since 1999, and by now approximately 700 graves have been excavated, along with some objects and crypts.

In this study, we have researched 425 graves excavated between 1999 and 2004. The basis of the anthropological analysis was the determination of sex and age of death, inclusion metric data, and paleopathological and taxonomical analysis. To determine these data, we have used common anthropological methods. Paleopathological and taxonomical examinations have been carried out using macromorphological methods, though in certain cases radiographical analysis was also applied.

After the determination of sex and age, we could establish that in this population the sex ratio was 50%-50%; the percentage of infants (INF1, INF2, JUV) was 49%, and elderly (SEN) 7%.

By means of the measurements of humerus, radius, ulna, tibia and femur we determined the height of people. The average of the height of adult males was 170 cm, adult females 160 cm; the highest was 181 cm both among males and females, the minimum height was 157.5 cm among males and 147.7 cm among females.

In accordance with general medieval health, many of the skeletons showed different forms of paleopathological lesions: periostitis, osteomyelitis, arthritis; minor developmental anomalies: sacralisation, lumbarisation, spina bifida, dislocation of the hip; traumas: fractures of humerus, radius, ulna, ribs or clavicle. There also were infectious bony lesions due to TB and syphilis. We found some metabolic disturbances of bone: osteoporosis; and circulatory and hematologic disorders as well: cribra orbitalia and cribra cranii.

The taxonomical analysis could yield some very interesting information because there is no such data available for this town. There are some finds in the area of a 'Kun' population (for example in Kiskundorozsma), and we suppose there was a Mongolid population in Szeged after the Hungarian conquest. For taxonomical analysis we have to research anatomical variations like sutura metopica, os Wormiana, torus palatinus, torus mandibulae and maxillae, fossa canina and several dental variations.

This presentation is for the preliminary results only.

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Mitochondrial sequence variation in ancient horses from the Carpathian Basin and possible modern relatives

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Whatever the place, ethnic changes always leave their footprint in the local culture and genetic makeup and the same applies to the different types of horses moving with their owners. This thesis is concerned with the mitochondrial control region genotypes of ancient and modern horses from the Carpathian Basin, where in the late 9th cenury the incoming pagan Hungarian tribes permanently changed the population.

Studies of mitochondrial DNA have shown that modern horses are descended from at least 77 different wild mares, with a last common ancestor over 300,000 years ago, and so probably inhabiting very different regions. Despite this matrilineal genetic diversity, correlations between modern breeds of horses and mitochondrial genotype are often uncommon. This is probably because horse-trading and horse-stealing, sometimes over long distances, have been popular and profitable for a long time.

To determine genetic diversity and origin of horse populations in the Carpathian Basin at the time of the Avars and of the Hungarian Conquest, mitochondrial DNA analysis was undertaken on 31 archaeological horse remains, excavated from authentic, well-dated Avar and pagan Hungarian burial sites. Based on a supposed relationship, modern Hucul and Akhal Teke horses were included in the analysis. To reveal relationships to other ancient and recent breeds, mtDNA sequences from 79 breeds representing 913 individual specimens were combined with our sequence data. Sequences were aligned and truncated to a length of 247 bp to accomodate published short sequences (nucleotide positions 15495-15740 of reference sequence X79547).

Estimation of standard diversity measures, such as haplotype diversity (h) and nucleotide diversity (π) were performed in DnaSP 4.50.2.

To compare our samples with other modern and ancient horse sequences, 921 previously published equine mtDNA CR sequences with fully overlapping standard 247 bp lengths were obtained from the database (http://www.ncbi.nlm.nih.gov/Genbank).

Median-joining network was constructed using the NETWORK 4.5.1 software (Fluxus Technology Ltd. at www.fluxus-engineering.com) to reveal approximate genealogical relationships among the haplotypes found in our ancient and recent breeds and the haplogroup-indicating sequences.

Both genetic distances and haploltype-based methods indicate a clear separation between horses of the Avar and Hungarian leading nobles. Avar sequences were genetically heterogeneous, closely related to Eastern breeds; (with Mongolian and north Russian Vyatskaya groups). This Asian relationship can also be seen in the genetic distance matrix and the haplotype network.

By contrast, beside the great heterogenity and unique haplotypes, the ancient Hungarian horses showed a relatively close relationship with the Turkoman Akhal Teke and Norwegian Ffjord. It confirms the assumption of Hecker (1955) and is an admixture to the clew up of the Hungarian origin. After a short communication of Bjørnstad (2003), different distance measures suggest genetic associations between northern European horse breeds and the Mongolian native horse. By all means, the high variability of Hungarian horse haplotypes may be connected with the well-attested, continent-wide raiding habits of the ancient Hungarians. The Hucul data show no such relationship. Our results show that the ethnic changes induced by the Hungarian Conquest in the late 9th century were accompanied by a similar change in the stables of the Carpathian Basin.

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Isolation and characterization of bacterium and phage isolates which have biocontrol ability against *Pseudomonas* strains pathogenic to *Pleurotus ostreatus*

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Pleurotus ostreatus is one of the most extensively cultivated mushrooms in the world; however significant loss of crop and quality arises from bacterial diseases causing by different bacterial pathogens. The yellowing of *Pleurotus ostreatus* and the brown blotch disease of *Agaricus bisporus*, caused by *Pseudomonas tolaasii* is well known (Lee and Cha 1998). The bacterium produces the toxins tolaasins that disrupt the cellular membrane by forming pores (Rainey et al. 1992).

Ps. tolaasii can be identify easily, with tolaasin toxin gene specific primers. This method is much more reliable than the white line test. The *Ps. tolaasii* is able to make a phenotypic switch; this variant form is nonpathogenic and differs from the wild type in a range of biochemical and physiological characteristics (Cutri et al. 1984).

Moreover other fluorescent pseudomonads such as *Ps. agarici*, *Ps. constantinii* and *Ps. gingeri* can cause various symptoms as well. The same degree of discoloration may be caused by dissimilar species of pseudomonads, suggesting that the factors are not exclusive to a particular pseudomonad species (Godfrey et al. 2001).

Many investigations have been carried out to find an appropriate method for preventing or controlling this disease. There are trials to use of chemical wash formulations, including such chemicals as calcium chloride, sodium hypochlorite, hydrogen peroxide, bronopol and antibiotics in watering mushrooms, but none of them has been found to be fully effective and non-toxic to humans (Wong and Preece 1985).

Biological control by competition has also been investigated. Potential bacterial control agents have been isolated and shown to be active antagonists (Nair and Fahy 1972).

In this study, the *Pseudomonas* strains of an infected oyster mushroom farm in Hungary were investigated. Sixty strains were isolated on Gould's S1 medium, which consistently gives high selectivity and good recoveries of fluorescent pseudomonads with samples obtained from a variety of habitats. S1 medium has several advantages over other media used for the isolation of fluorescent pseudomonads. The identification of the strains was carried out by sequencing a part of the *rpoB* gene or in some other cases a part of the 16S rDNA gene. The *rpoB* gene codes for the RNA polymerase β-subunit, it is a highly conserved essential gene, so it could be used for bacterial identification. The pathogenicities of the strains were tested on yeast extract media in Petri-dishes, in direct confrontation tests.

To find and effective antagonistic agent, we isolated and evaluated fluorescent pseudomonads, bacilli and lytic bacteriophages, against *Pseudomonas tolaasii*, and other pathogenic *Pseudomonas* strains.

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Interacting protein partners on Drosophila telomeres

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The *proliferation disrupter* (*prod*) gene in *Drosophila* encodes a 346- amino-acid protein that localizes strongly to the centric heterochromatin of the second and third chromosomes as well as to >400 euchromatic sites, and all telomeres. In *Drosophila melanogaster*, three telomeric domains can be distinguished by DNA sequence and by proteins associated with them: the end of the chromosomal DNA molecule (capping complex), the retrotransposon array consisting of three non-long terminal repeat retrotransposons, *HeT-A*, *TART*, and *TAHRE* (HTT), and the subtelomeric repetitive telomere associated sequences, repetitive region (TAS). Chromosome length in *Drosophila* is maintained by targeted transposition of the three telomere-specific non-long terminal repeat retrotransposons, HTT, to the chromosome end.

Immunofluorescence stainings of different mutant telomeres clearly showed, that Prod binds to the *HeT-A* element of the HTT array. We could also show that Prod binding represses *HeT-A* transcription, nevertheless does not influence HTT length. Reduction of Prod levels in heterozygous $prod^{k08810}$ null mutant flies results in elevated levels of *HeT-A* transcripts in ovaries as well as in third instar larvae, while it has no effect on genomic *HeT-A* copy number, which we used as a measurement for HTT length.

To identify proteins that may interact with Prod, we performed a yeast two-hybrid screen, using the Clontech Matchmaker cDNA library and the entire *prod* cDNA as bait. About 120 000 cDNA clones were tested and about 100 potential Prod interactors were identified. Prod was found 4 times, suggesting that Prod interacts with itself. We also identified Z4 as one of the interacting proteins, which is one of the few proteins known to be associated with the HTT telomeric domain. The Chromator protein, which has previously been shown to co-immunoprecipitate with Z4 and co-localize with Z4 in interbands of polytene chromosomes and at some telomeres, was also found to interact with Prod. We could verify the latter interaction with co-immunoprecipitation experiment.

Proteins Uba2 and Lesswright were the strongest interactors of Prod in the yeast two hybrid screen.Uba2 is the E1 SUMO activating enzyme regulating the initial steps of sumoylation, - a posttranslational protein modification system modifying protein activity - while the second step is performed by the E2 ubiquitin-conjugating enzyme encoded by the lesswright gene in *Drosophila*. Computer sequence analysis showed that Prod (and Chromator) have potential sumoylation sites. This raised the possibility that either Prod itself is sumoylated or sumoylation enzymes are recruited by Prod to sumoylate Prod-associated proteins. Our immunostainings confirm this notion, demonstrating that most if not all sumoylated proteins on telomeres are located on the HTT array.

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We tried to identify the sumoylation site(s) in Prod by disrupting each potential site one by one, and expressing the HA-tagged mutant proteins in transfected S2 cells. Next we immunoprecipitated the mutant proteins with anti-HA antibody, and tested their molecular weight and sumoylation on Western blots. In one of the mutant proteins the high molecular weight sumoylated band seemd to disappear, and the S2 cells expressing this protein showed an altered Prod chromosomal immunostaining pattern. This indicates that the Prod protein is sumoylated at the 123rd lysine aminoacid.

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The role of the small GTPase LIP1 in the function of the plant circadian network

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The circadian clock is a biological timing mechanism that provides rhythmicity to gene expression, metabolism, and physiology with ~24h periodicity. The central oscillator of eukaryotic clocks is based on the network of clock genes and proteins, which are interconnected by transcriptional/translational negative feed-back loops.

Current models of the plant circadian clock postulate three interlocked feedback loops. A pair of single Myb-domain transcription factors, *CIRCADIAN CLOCK ASSOCIATED1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)*, plays central roles in two loops. In one loop, *CCA1* and *LHY* repress the expression of the Pseudo-Response Regulator gene *TIMING OF CAB EXPRESSION 1 (TOC1). TOC1* closes the first loop by inducing *CCA1* and *LHY* transcription for the next cycle. In a second loop, *PRR7* and *PRR9,* are induced by *CCA1* and *LHY*. *CCA1* and *LHY* are subsequently repressed by *PRR7* and *PRR9*. In a third loop, *GIGANTEA (GI)* and, possibly, *PRR5* are positive regulators of *TOC1*. *GI* is negatively regulated by both *CCA1/LHY* and *TOC1* (McClung 2008).

The *lip1-1* (light insensitive period 1) mutant isolated from *Arabidopsis thaliana* displays novel circadian phenotypes. *lip1-1* was isolated as an early-phase mutant based on the expression pattern of *CAB2:LUC* circadian output marker in constant darkness. In wild-type plants, period length shortens with increasing light fluence rates and the phase of rhythms can be shifted by light pulses administered to darkadapted plants. In *lip1-1*, period length is nearly insensitive to light intensity and larger phase shifts can be induced during the subjective night (Kevei et al 2007).

The first aim of our work was to determine the molecular mechanism by which LIP1 affects the plant circadian clock. Transcript levels of clock genes were determined by quantitative real-time PCR in *lip1* mutants. Our data show that LIP1 affects the expression of *GI*, *PRR9* and *TOC1*. The effect on *GI* expression was supported by the analysis of *gi-lip1* double mutant plants.

We generated promoter:LUC+ reporter gene constructs for each core clock genes in *lip1* mutant background and we could prove that the transcription of all core clock components is affected by the mutation.

Our second aim was to identify how the function of LIP1 is controlled. LIP1 is a plant-specific atypical small GTPase. Small GTPases are molecular switches shuttling between the GDP-bound inactive and the GTP-bound active states. For this process they require downstream signaling elements (effectors) and upstream signaling elements (e.g. GEFs) (Berken et al 2005). We found that LIP1 interacts with a member of the plant specific family of RopGEFs, RopGEF7 in yeast two-hybrid system. However, the insertion mutant allele of *RopGEF7* showed no circadian phenotype in planta. The family of RopGEFs consists of 14 members. We tested the circadian phenotype of insertion mutants for all of them and found that a mutant allele of *RopGEF2* has a *lip1*-like circadian phenotype. *lip1* mutant plants show stress phenotype also, they are sensitive to salt. *RopGEF2* mutant plants display a *lip1*-like salt phenotype. RopGEF2 might be the member of the RopGEF family which promotes LIP1 function.

Previous data showed that LIP1 is localized in the cytosol, nucleus and in cell compartments as well. We tested the function of *nuclear export signal* (NES) or *nuclear localization signal* (NLS) tagged YFP-LIP1 fusion proteins in *lip1* mutant background to see if any of the *lip1* phenotypes could be complemented. YFP-LIP1-NLS fusion proteins could restore the circadian phenotype. Neither of the constructs could restore the salt sensitivity phenotype. These data indicate that LIP1 affects the circadian clock in the nucleus, but nucleo-cytosolic shuttling is required to fulfill its role in tolerating salt stress.

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Progress report. Investigating the neuroprotective function of Hsp27

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Hsp27 is expressed in many cell types and tissues at specific stages of developement and differentiation. It is an ATP independent chaperone, its main function is to bind misfolded proteins hereby block the formation of protein aggregates. The protein can protect cell against stressful stimuli through its several other properties. For example it has been shown to modulate apoptosis since it can interact with and inhibit key components of the apoptotic signaling pathway. Hsp27 can also increase the resistance of cells to oxidative injuries as it reduce lipid peroxidation, protein oxidation and f-actin architecture disruption.

In order to study the neuroprotecive effects of Hsp27 in vivo, transgenic mouse lines overexpressing the human Hsp27 were generated. The human Hsp27 protein expressed at high level in the brain of the transgenic mice demonstrated by western blot analysis. The cellular localization of the expression of transgenic protein in the brain was detected by immunohistochemistry. We found strong expression of the transgene in the cerebellum, hippocampus and cerebral cortex.

The neuroprotective effect of Hsp27 protein was investigated in acute and chronic ethanol administration. In the acute administration studies animals received an intraperitoneal injection of 20% ethanol (2g/kg) than behavioural studies were performed to analyse motor coordination and muscle strength. Five different behavioural tests were performed and in three of the tests statistical analysis using one-way analysis of variance (ANOVA) revealed a significant difference in the performance of transgenic and wild type mice. In the inverted screen test all of the wild type animals showed ataxia compared to the transgenic group, where it was significantly less (57%) (p=2,925E-4). In the beam walking test 86% of wild type mice fell off the beam, while in the transgenic group only 53% (p=0,031). In the footprint analysis we found significant difference in the stride length between the transgenic and wild type groups (p=0,002 for the forlimb and p= 5,6036E-6 for the hindlimb).

In the chronic ethanol treatment drinking water was replaced by a 20% of ethanol solution for five weeks. At the end of the treating period 10 μm frozen sections were made from the brain of the animals. Neurodegeneration was detected by Fluoro-Jade C staining. We detected less degenerated neurons in the brain of the transgenic mice compared to wild type mice especially in the cerebellum, hippocampal region and cerebral cortex.

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The role of dADA2b adaptor proteins in dSAGA histone acetyltransferase complex

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In eukaryotes the genetic material is present in a compact chromatin structure consisting of DNA and histone proteins. Histone acetyltransferase (HAT) complexes play a role in chromatin structure modifications which might lead to changes in gene expression. The GCN5 protein is the catalytic component of several multiprotein HAT complexes which modify chromatin structure by acetylating specific lysine residues at the N-terminal tails of histone H3 and H4. Many of the GCN5-containing HAT complexes also contain ADA-type adaptor proteins, which play roles in modulating HAT activity and specificity.

In *Drosophila melanogaster* our group has described two ADA2 proteins (dADA2a and dADA2b) in two GCN5-containing HAT complexes, ATAC and dSAGA which have different histone specificities. The dADA2b-containing dSAGA complex is involved in the post-translational modification of nucleosomal histone H3 at lysine (K)9 and K14. Furthermore, analysis of the *dAda2b* gene revealed that by alternative splicing it gives rise to two mRNAs (dAda2bS and dAda2bL). Despite the detection of two different forms of dAda2b message in earlier studies, the production and function of the two protein isoforms have not been studied.

In my thesis work I have shown that *dAda2b* produces at least two protein isoforms, dADA2bL and dADA2bS during the course of development and demonstrated that dADA2b proteins are most abundant in pupae, in the developmental stage when in the absence of the proteins *dAda2b* null mutants die.

My further studies included generation of *dAda2b* mutations which eliminate the production of either or both dADA2b isoforms in order to characterize dADA2b function in dSAGA complexes. Functional studies of *dAda2b* null mutant reported so far demonstrated the essential function of *dAda2b* gene in Drosophila development and the requirement for histone H3K9 and K14 acetylation. Genetic studies showed that neither dADA2b isoform alone could provide a complete restoration of *dAda2b* function, suggesting that both are required for development. On the other hand, either dADA2b isoform can productively participate in dSAGA complexes and render those at least partially active in histone H3 acetylation.

To study the molecular consequences of the loss of H3 K9 and K14 acetylation we compared the mRNA profiles of wild-type and *dAda2b* mutant animals in late L3 larval and in P4 pupal stages by cDNA microarray. Global gene expression profiling indicates that the

expression of less than 5% of the genes is significantly changed in *dAda2b* null mutants at both time points analyzed. We observed a relatively small overlap between the two stages in both mutant and wild-type samples. More than half of the affected genes are up-regulated, of which the high number of defense-related genes is particularly striking.

To demonstrate whether dSAGA has a direct role in the regulation of those genes which showed altered transcription levels in *dAda2b* mutant, we performed chromatin immunoprecipitations (ChIP) to detect the presence of dSAGA-specific histone H3 acetylation marks on these genes. Interestingly, we found that *dAda2b* mutation affects the H3K9ac level by a different extent at dSAGA-dependent and independent genes. In *dAda2b* mutants the histone acetylation levels are decreased both at dSAGA up-regulated, and at dSAGA down-regulated genes, while in the promoters of dSAGA independent genes a high level of histone H3K9ac is maintained.

Our data support the notion that different dADA2 proteins contribute to the functional versatility of multiprotein complexes and demonstrate that the loss of dSAGA specific H3 K9 and K14 acetylation affects (up- or down-regulates) the expression of a rather small subset of genes but does not cause a general transcription deregulation. We envision that the loss of *dAda2b* function results in a global decrease in H3 acetylation which can lead to an increase or to a decrease in the transcription level of genes.

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