

## DISSERTATION SUMMARIES

### DNA Replication across the protein-DNA adduct

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In cells, DNA is tightly associated with a variety of proteins that serve both to maintain the structural organization of the genetic material and to coordinate cellular processes including replication, repair, recombination, and transcription. Many endogenous compounds (e.g., metabolites of lipid peroxidation) as well as environmental agents are reactive with both DNA and proteins and thus can produce covalent linkage between these two types of macromolecules.

DNA-protein cross links (DPCs) arise in biological systems as a result of exposure to a variety of chemical and physical agents, many of which are known or suspected carcinogens. These DPCs formed within the cells are usually removed/ cleaved by different cellular mechanisms. The unresolved DPCs can hinder normal functioning of a cell by blocking regular cellular mechanism like DNA replication, transcription and others.

Despite the recognition of the biological significance of DPCs, there are very limited data concerning the repair of these lesions. One possible hypothesis is that the covalent or irreversible bondage of a protein to DNA somehow modifies the whole structure of DNA double helix and hence allowing cell to recognize these DPCs as unnatural nucleotide base pair. The mechanism how a cell recognizes these DPCs and how these unnatural structures are resolved still remain to be unclear.

Analyses of data generated in prokaryotes revealed the existence of mechanisms of active DPC removal and suggested that more than one repair pathway can be involved in the repair of these lesions. There are couple of possible hypotheses, one being the protein part of the DPCs is to be degraded/ cleaved specifically by a protease, and other Nucleotide excision repair (NER), the repair mechanism in which a damaged base is cleaved and replaced by a regular nucleotide bases. However all the hypotheses lack a proper experimental system. It has been previously reported that DNA replication machinery fails to replicate the DNA in the presence of DPCs revealing the fact of stalling the DNA replication fork at the Site of DPCs. However the exact mechanisms how an ongoing replication fork can bypass these DPCs is largely unknown due to lack of a proper in-vivo or in-vitro experimental system.

In the present study we are developing an in-vitro system to monitor stalling or bypass of DNA replication machinery at the site of DPCs. To accomplish the above task a suicidal DNA substrate is designed to trap a protein irreversibly. DNA binding or DNA modifying proteins can be used to crosslink to the DNA of known sequence. This cross-linked DNA-protein substrate is further purified and can be used as a template for the DNA replication. By using different DNA polymerase including some of the specialized TLS (translesion synthesis) polymerase which specifically replicates damaged DNA; it is possible to check bypass of these DPCs. In future these experiments will also reveal whether a specific polymerase is involved to resolve this kind of naturally occurring cross links.

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### Data to the analysis of paleopathology of the Medieval Age in the region between the Danube and Tisza rivers (preliminary report)

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Human paleopathology can be defined as the study of diseases in ancient populations by the examination of human remains (dry skeletons and mummies). However, the anthropological study of diseases in antiquity is very complex and challenging. The interplay of many variables – host resistance, pathogen virulence, cultural practices, ecological settings, malnutrition, crowding – needs to be considered.

The aim of the investigation is to perform a complete comparative analysis of populations dated to the 11th-17th centuries in the region between the Danube and Tisza rivers based on the presentation and evaluation of the paleopathological alterations.

The following series were included in this study: Nyárlőrinc-Hangár utca, Kalocsa-Szentháromság tér, Kalocsa-Belvárosi Iskola,

Bácsalmás-Mosztonga, Dunapataj-Szent Tamás domb. The samples contain the remains of 756 individuals (163 males, 54 females, 207 undetermined, 332 subadults). This skeletal material is collected at the Department of Anthropology, University of Szeged.

The specimens have been analysed for the determination of the age at death and sex and scored for the measurements. Concerning the pathological conditions, the macro-morphological examination was completed - in some cases - with radiological analyses. In one case the molecular analysis was carried out to estimate the DNA of *Mycobacterium tuberculosis*. (This investigation was made at the München University - Institute of Pathology.)

The following disorders have been identified: traumatic lesions, specific and non-specific infections, haematological anomalies, joint diseases, bone-tumor and tumor-like anomalies, developmental disorders, and enthesopathies.

It is the most important to highlight the cases of skeletal tuberculosis (one case) and –syphilitic lesions (two cases) (Nyárlőrinc-Hangár utca; Pálfi et al. 1997; Balázs et al. 2005), for these diseases were among the most important selective factors in human populations in antiquity. In the sample Dunapataj-Szent Tamás domb, the frequency of the developmental anomalies is very significant by the reason of endogamy (Balázs and Marcsik 2007b).

In the Nyárlőrinc-Hangár series (11th-17th centuries; V. Székely 1987), there was excavated a partly mummified foetus which was buried in a crock at the edge of this cemetery and dated to 19th century on the basis of a copper coin which was put into the crock (Balázs and Bölkei 2007a).

This presentation is only a preliminary result.

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## Functional characterization of the plant SET protein: from phosphatase inhibition to heat stress tolerance

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Even small environmental changes can induce expression or repression of hundreds of genes in plants, contributing to their endless adaptation to the changing environment. Regulation of such a synchronized genomic event has to employ chromatin remodelling – a process that involves post-translational modifications of histones. One of the putative proteins involved in the regulation of histone modification patterns is SET. SET, belonging to the NAP/SET family of potential histone chaperones, is a multifunctional protein involved in very diverse cellular processes in mammals.

It was previously shown that human SET inhibits protein phosphatase 2A (PP2A) (Li et al. 1996), a major serine/threonine phosphatase both in plants and animals. It was also demonstrated that SET is associated with transcriptionally active loci in response to heat shock in *Drosophila melanogaster*, and these regions encoding heat shock proteins are marked with phosphorylation of histone H3 at serine 10 (Nowak et al. 2003).

Although the members of the NAP/SET family are well characterized proteins in animals (reviewed in Park and Luger 2006), we have little information on the plant NAP1 (nucleosome assembly protein1)-related proteins. The aim of our studies was hence the characterization of the *Arabidopsis* SET protein.

Our results revealed that the recombinant *Arabidopsis thaliana* SET protein exhibited inhibitory effect on the activity of purified preparations of rabbit PP2A and PP1 (protein phosphatase 1) catalytic subunits against a phospho-histone substrate. In addition, purified SET inhibited the dephosphorylation of histone H3 at serine 10 position by immunoprecipitated *Arabidopsis* PP2A and interacted *in vitro* with purified calf histone H3.

Phosphorylation of serine 10 on histone H3 is coupled with two opposite chromatin states: it is associated with mitotic chromosome condensation, while it occurs also during interphase in correlation with transcriptionally active loci (Johansen and Johansen 2006). Since our results suggest that SET may have a role in the maintaining of this kind of histone modification in plants, we propose a role for SET in transcriptional regulation. The verification of the involvement of the *Arabidopsis* SET in gene expression control, however, needs further investigations.

We also demonstrated that the subcellular localization of SET was influenced by a heat stress treatment at 45°C. In response to heat, SET accumulated in the nucleus, while under standard conditions it is located predominantly in the cytosol. Interestingly, other types of stresses including heat stress at lower temperature (37°C), salt stress, heavy metal stress or genotoxic stress did not cause the nuclear accumulation of SET, suggesting a specific role for SET in certain plant stress responses.

Taken together, the *Arabidopsis* SET protein is a potent inhibitor of animal and plant phosphatases and may have a role in heat shock tolerance as indicated by its altered (nuclear) localization in response to a 1h 45°C treatment. Thus, in the light of our results we can presume that the investigation of SET can be of practical importance, since it might have a role in the stress tolerance of plants. This hypothesis is currently investigated in SET-overexpressing transgenic plants.

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## Cross-talk between cannabinoid CB<sub>1</sub> and GABA<sub>B</sub> receptors in rat brain hippocampus

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Cannabinoid CB<sub>1</sub> and the metabotropic GABA<sub>B</sub> receptors have been shown to display similar pharmacological effects and co-localization in certain brain regions. Previous studies have reported a functional link between the two systems. As a first step to investigate the underlying molecular mechanism, here we show cross-inhibition of G-protein signaling between GABA<sub>B</sub> and CB<sub>1</sub> receptors in rat hippocampal membranes. The CB<sub>1</sub> agonists R-Win55,212-2 displayed high potency and efficacy in stimulating Guanosine-5'-O-(3-[<sup>35</sup>S]thio)triphosphate, [<sup>35</sup>S]GTPγS binding. Its effect was completely blocked by the specific CB<sub>1</sub> antagonists AM251 suggesting that the signaling was via CB<sub>1</sub> receptors. The GABA<sub>B</sub> agonist baclofen and SKF97541 also elevated [<sup>35</sup>S]GTPγS binding by about 60%, with potency values in the micromolar range. Phaclofen behaved as a low potency antagonist with an ED<sub>50</sub> ≈ 1 mM. However, phaclofen at low doses (1 and 10 nM) slightly but significantly attenuated maximal stimulation of [<sup>35</sup>S]GTPγS binding by the CB<sub>1</sub> agonist Win55,212-2. The observation that higher concentrations of phaclofen had no such effect rule out the possibility of its direct action on CB<sub>1</sub> receptors. The pharmacologically inactive stereoisomer S-Win55,212-3 had no effect either alone or in combination with phaclofen establishing that the interaction is stereospecific in hippocampus. The specific CB<sub>1</sub> antagonist AM251 at a low dose (1 nM) also inhibited the efficacy of G-protein signaling of the GABA<sub>B</sub> receptor agonist SKF97541. Cross-talk of the two receptor systems was not detected in either spinal cord or cerebral cortex membranes. It is suggested that the interaction might occur via an allosteric interaction between a subset of GABA<sub>B</sub> and CB<sub>1</sub> receptors in rat hippocampal membranes. Supported by NKTH DNT 08/2004 and OTKA TS 049817 research grants.

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## Functional analysis of *Drosophila melanogaster* histone H4 specific acetylase complex and its role in regulating chromatin structure

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Numerous enzymes and protein complexes are known to bring about changes in the state of chromatin by different mechanisms with resultant effects on gene expression. One class of complexes including the yeast SWI/SNF and a number of others from various organisms, alter the DNA packaging in an ATP-dependent manner. Another class of chromatin structure regulating factors acts by covalently modifying histone proteins. The various modifications include phosphorylation, ubiquitination, ADP-ribosylation, methylation, sumoylation and frequently acetylation, catalyzed by histone acetyltransferases (HATs). In many cases HAT enzymes are components of complexes which also contain among others, ADA-type adaptors.

Recently our laboratory, in parallel with several others, has showed that contrary to the single ADA2 adaptor protein present in *Saccharomyces cerevisiae*, different GCN5-containing HAT complexes of *Drosophila melanogaster* cells contain two related ADA2 proteins encoded by genes referred to as *dAda2a* and *dAda2b*. In several other metazoan organisms, including mouse, human and *Arabidopsis*, there

are also two ADA2-type coactivators. Biochemical separation of ADA2-containing *D. melanogaster* complexes indicated that dADA2a is present in a smaller (0.8 MDa) and dADA2b in a larger (2MDa) complex which corresponds to the *Drosophila* homologue of yeast SAGA complex. In a number of independent studies it was shown that in the absence of dADA2b or dGCN5, in other words, in the absence of functional SAGA, the acetylation of histone H3K9 and K14 is greatly reduced, while the H4K8 acetylation is not affected.

In this work we provide evidence that the dADA2a protein is a specific component of the smaller *Drosophila* HAT complex which during the course of this work became identified as ATAC. We demonstrate the genetic interaction between *dAda2a* and *dGcn5* genes and show their role in H4 acetylation. Finally, we describe the functional interplay between components of the ATAC complex and ATP-dependent nucleosome remodeling ISWI-containing NURF complex.

We provide several lines of evidence for the functional linkage between dADA2a and dGCN5. We show their physical and genetic interaction by yeast two hybrid assays and by analyzing the phenotype of specific single and double mutants, respectively. The loss of either *dGcn5* or *dAda2a* function results in similar chromosome structural and developmental defects. *dGcn5/dAda2a* double-null mutants or a combination of *dAda2a* and *dGcn5* hypomorph alleles result in a phenotype stronger than that of either of the two mutations alone. The overexpression of dGCN5 protein by the use of an act-GAL4 driver in *dAda2a* mutant background results in a partial rescue. Furthermore, the phenotypic features of *dAda2a* mutants indicate a developmental block at the time of larva-pupa transition similarly as it was shown by others for *dGcn5* mutants. In accord with this, by analyzing the puff formation at sites containing ecdysone induced genes and using RT-PCR and Q-PCR to measure specific mRNA levels we demonstrate that the expression of several ecdysone-induced genes such as BR-C, Eip74 and Eip75 are downregulated in the absence of dADA2a protein.

Immunostaining of *Drosophila* polytene chromosome and Western blot analysis revealed a significantly decreased level of K5 and K12 acetylated histone H4 in *dAda2a* and *dGcn5* mutants, while the acetylation established by dADA2b-containing GCN5 complexes at H3K9 and K14 was unaffected. These results, for the first time in the literature, clearly establish the *D. melanogaster* ATAC as a histone H4-specific HAT complex.

In a set of independent experiments we showed functional interaction between the histone modifying ATAC and the nucleosome remodeling NURF complex. Using appropriate mutants strains we showed that there is genetic interaction between genes encoding ATAC subunits and the NURF subunit ISWI. In addition, immunostaining of polytene chromosomes with dADA2a-specific Ab revealed that the ADA2a binding to *Iswi* chromosomes was strongly reduced. In agreement with this data, immunoblot analysis and chromosome immunostaining showed a significant decreased of K12 acetylated H4 level of salivary gland polytene chromosomes of *Iswi* and *Nurf301* mutants.

Taken together, these results strongly suggest a functional interaction of nucleosome remodeling and histone acetyltransferase complexes. Our data demonstrate that the function of NURF complex is required for the binding of ATAC to chromatin and for subsequent acetylation of H4K12 residues.

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## Study of *Medicago truncatula* RRK1 receptor-like cytoplasmic kinase interacting proteins

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Small GTP-binding proteins of the Rho family play a role as regulators of signal transduction in plants. These proteins called ROP („Rho of plant”) participate in key cellular events including the determination of polar growth, vesicular trafficking, stress and hormone responses or cell wall synthesis. ROPs act as molecular switches cycling between a GDP-bound inactive and a GTP-bound active state. In our group an alfalfa receptor-like cytoplasmic kinase, termed RRK1, has been identified by yeast two-hybrid screen as an interacting partner of the active MsROP3 GTPase. RLCKs have no extracellular and/or transmembrane domains and are localized in the cytoplasm. The function of the RLCKs is not well understood; they have hypothetical roles in RLK-dependent signaling. Our finding was among the first indications that Rop GTPases may directly influence kinase activity in plants similarly as in animals.

In order to identify downstream signaling events of RRK1, our group applied the yeast two-hybrid system with a cDNA library made from 4-day-old root nodules on *Medicago truncatula* roots, using RRK1 as bait. Several clones were identified and sequence analyzed. The sequence comparison revealed that one of our clones carries a plant specific guanine nucleotide exchange factor (GEF) domain. Conversion of Rops from the inactive GDP-bound to the active GTP-bound form is catalyzed by GEFs. In *Arabidopsis*, the ROPGEF family has 14 members, which contain a plant-specific central, highly conserved catalytic domain termed PRONE (Plant Specific ROP Nucleotide Exchanger) or formerly DUF315, and variable N- and C terminal regions.

Why is so important to have a kinase that is capable to interact with a ROP GTPase as well as a ROPGEF? GEF proteins have the potential to transfer signals from receptors to ROP GTPases. A huge family of receptor-like kinases (RLKs) has been found in plants but their downstream signaling events are hardly known. Similarly, it is not known what are the upstream signaling steps resulting in ROP activation. What we currently know is that a tomato protein called KPP (kinase partner protein) has been identified as binding partner of the cytosolic domains of the pollen-specific RLKs, LePRK1 and LePRK2. This KPP protein is a homolog of *Arabidopsis* ROPGEF1 and is phosphorylated *in vitro* by LePRK1. Our results indicate that a further type of kinase (RLCK) might be involved linking ROP- and RLK-mediated signaling pathways.

In order to prove this hypothesis, as a first step, we showed the interaction between the MtGEF and MsROP3 proteins. In our yeast two-hybrid experiments, MtGEF displayed strong interaction with the non-nucleotide bearing wild-type and the constitutive active (CA) mutant of MsROP3. Wild type, CA- and dominant negative (DN) mutants of MBP-fused MsROP3 and His tagged-MtGEF fusion proteins expressed in *E. coli* were used for pull down assay. With this *in vitro* protein-protein interaction assay we were able to confirm our yeast results. Then the expression level of MtGEF was investigated in different *Medicago truncatula* tissue types by QRT-PCR, but it showed very low expression in almost all tissues therefore a correlation with MsROP3 or RRK1 expression could not be made. Recently, the full length MtGEF cDNA sequence has been amplified by PCR from a *Medicago truncatula* cDNA library and cloned into various expression vectors. In the future we would like to confirm our previous observations with this full length form as well as to further characterize the potential signaling interactions. This will include the determination of GEF activity toward MsROP3 and the RRK1 kinase activity towards MtGEF. We suppose that MtGEF could be an elusive link between RLKs and ROPs in a plant-specific signal transduction mechanism that also includes a ROP-dependent feedback regulation of GEF activity through RRK1.

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## Phylogeny of Alloxysta (Hymenoptera, Cynipoidea, Figitidae, Charipinae) species – morphology vs. molecules

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Members of the figitid genus *Alloxysta* (Förster 1869) are parasitoids of hymenopteran natural enemies of economically important aphid species. Therefore these hyperparasitoid species have large impact on the biological control of insect pests. Due to their minute size most of the morphological characters which are widely used in the taxonomy of other cynipoid taxa, are variable and highly reduced. Most of the species are hardly distinguishable morphologically and it is impossible to determine if the variability is intra- or interspecific. According to some authors there are only a few, very variable and generalist *Alloxysta* species whereas others suggest that the genus contains much more species which are less variable but more specialized. Current phylogenetic relationships of the genus are based on the same, often questionable morphological characters. So far no studies were carried out using molecular markers determining species limits and resolving the phylogeny of the genus. 20 morphological characters were widely used for *Alloxysta* species determination. On the basis of three characters: presence of the propodeal carina, pronotal carina, radial cell, the genus might be divided into six species groups. Mapping morphological characters on a molecular-based phylogeny enabled examination of character evolution. In this study, 20 morphological characters from western Palearctic *Alloxysta* were mapped on a phylogenetic tree reconstructed from region of the cytochrome-c-oxidase I (COI) and the ribosomal 28S D2 genes analysed with parsimony Bayesian, maximum-likelihood and distance based methods. The COI and 28S D2 trees were congruent. The above mentioned morphological characters may have evolved in parallel in different species groups of *Alloxysta* and, taken alone, may be unsuitable for a subgeneric division of the genus, however, are suitable for species differentiation.

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## The effects of drought on changes in photosynthesis, hormone levels and grain yield in wheat (*Triticum aestivum* L.)

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Wheat is one of the main crops consumed by humans and it is cultivated in different environments. Under the temperate zone early-summer droughts are increasingly frequent and limit grain yield since they coincide with the grain filling period. There are several physiological traits related to water stress, and scientists make considerable effort to find direct correlations between these parameters and grain yield to facilitate the selection of cultivars for drought tolerance.

Photosynthesis is one of the main metabolic processes determining crop production. Chlorophyll fluorescence is a tool for monitoring the function of the photosynthetic apparatus, changes in response to water stress. The effect of drought on photosynthesis has long been a controversial subject and it is still not clear whether chlorophyll fluorescence parameters are good indicators for drought sensibility (Flexas et al. 2002). The plant hormone abscisic acid (ABA) plays a major role in plant responses to drought stress, facilitating plant survival (Zahng et al. 2006).

A comparison was made between changes of the parameters mentioned above, in seedling stage under osmotic stress and in reproductive growth phase under soil drought in two Hungarian (*Triticum aestivum* L. cv. MV Emese (resistant) and GK Élet (sensitive)) and two internationally known (*Triticum aestivum* L. cv. Plainsman (resistant) and Cappelle Desprez (sensitive)) wheat cultivars.

Our object was to compare the effects of osmotic and drought stress to find correlation between these treatments, and to compare the effects of water deficit on different physiological parameters, hormone levels (ABA and cytokine), grain yield and storage protein content in tolerant and sensitive varieties in the grain filling period. The water status parameters, CO<sub>2</sub> assimilation, chlorophyll *a* (*chl<sub>a</sub>*) fluorescence, pigment content and hormone levels were determined as a function of the development under osmotic stress in seedling stage (from germination to the 21<sup>st</sup> day after germination) and under water deficit in the grain filling period (from booting stage to the 24<sup>th</sup> day after anthesis).

Our results suggest that the photosynthetic parameters measured under osmotic stress are not comparable with those measured in flag leaves in the grain filling period. Different genotypes showed unique diversity in changes of these parameters, but common tendencies between the tolerant or sensitive cultivars were not found.

Pre- and post-anthesis soil drought did not result in characteristic modifications in PS II photochemistry of flag leaves in dark and light-adapted leaves, demonstrating that in this experiment these parameters did not correlate with sensitivity. Plants showed early senescence under water deficit. We found that sensitivity of the generative organs could be responsible for the higher decrease in grain yield. Changes of the ABA levels in the kernels showed a differing tendency: sensitive genotypes maintained high hormone levels, which can be unfavourable for grain growth. The different storage protein fractions of the mature grains were not significantly modified by drought, which confirm earlier results (Panozzo et al. 2001), but the gliadin to glutenin ratio increased significantly in one of the tolerant varieties.

Our results indicate that when the sensitivity of a genotype to drought stress are defined whole plants responses have to be taken into consideration. Responses of the vegetative and generative organs can be different and sensitivity of the generative phase and the fertilization process to water deficit may overwrite the efficient acclimation of vegetative organs.

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## Microarray and interaction network based identification of genes involved in germ cell development in *Drosophila melanogaster*

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Embryonic germ cell development of fruit fly (*Drosophila melanogaster*) depends on the germ plasm, the most posterior part of the egg cytoplasm. The germ plasm contains all factors which are necessary to induce germ cell fate. It has a characteristic distribution of proteins and contains a large number of localized RNA species, too (Williamson et al. 1996). Certain gene products being present in germ plasm might play crucial roles in germ cell determination and its subsequent development such as the germ cell migration, the passage through the embryonic midgut, and gonad formation. *Drosophila* is one of the most accepted model organism of germ cell research in the post sequencing era since numerous large *Drosophila* genomic databases are available for researchers.

We have developed and apply a microarray-based method to identify germ plasm enriched RNA-s. We performed a series of experiments on different microarray platforms to compare the RNA content of numerous germ plasm-less, germ plasm overexpressing and wildtype conditions. Collating our datasets with the list of the known germ plasm enriched transcripts, we found that germ plasm overexpressing vs. wild type comparison is the most appropriate method to identify new germ plasm enriched transcripts. In such comparisons 380 transcripts showed at least four times increase in germ plasm overexpressing condition. These transcripts were chosen for further analysis to confirm their germ plasm localization by making use of fluorescent RNA in situ hybridization (Lecuyer et al. 2007) on early *Drosophila* embryos. To be able to accomplish such a large number of in situ hybridisations we have developed a suitable PCR based single strand DNA labeling method.

Another approach we used, is a network based identification of novel germ plasm factors. We built up and investigated a germ plasm specific gene interaction network. First, we searched RNA localization databases (BDGP, Fly-FISH) and original publication for genes whose transcripts are exclusively or highly enriched in the germ plasm (Szuperák et al. 2005). This way, 136 as we called "original" germ line specific genes were found. Then we identified their primary genetic and yeast two-hybrid interactors by using the BioGRID database. Based on the GEO database, those primary interactors which are not expressed at early embryonic stages were filtered out. Finally, we constructed a gene interaction network which indicates all known interactions (325) among the original germ line specific factors (136) and primary interactors (325). We assume that the number of interactions of a given gene may mirror its importance in the network. Genes with large number of interactions, also called hubs, can refer to a central role of a given gene that have a good chance to show phenotype when it is mutated. We confirm this hypothesis by RNAi induced phenocopy analysis. We are currently analyzing the germ line specific phenocopies of a representative group of hubs as well as of the low connectivity control genes. The phenocopy of the RNA silencing is followed by the time laps video microscopy which allows distinguishing different type phenocopies: the complete absence or decreased number of germ cells, or its migration defects.

BDGP, Patterns of gene expression in *Drosophila* embryogenesis. <http://www.fruitfly.org/cgi-bin/ex/insitu.pl>

BioGRID, General repository for Interaction Datasets. <http://www.thebiogrid.org/>

Fly-FISH, A Database of *Drosophila* embryo mRNA localization patterns. <http://fly-fish.cabr.utoronto.ca/>

GEO: Gene Expression Omnibus. <http://www.ncbi.nlm.nih.gov/geo/>: GEO Accession: GSE3955

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## Oxidative stress, intrauterine retardation, modes of delivery

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Oxidative stress arises when the balance between oxidants and antioxidants is disturbed. The source of free radicals is the unpaired electron of molecular oxygen, which makes it unstable and electrically charged. In the lack of antioxidant molecules and enzymes, free radicals target lipids, proteins and DNA. Oxidative damage to DNA is a result of interaction of the nucleic acid with hydroxyl radical that generates strand breaks on the DNA. Oxidative stress is a physiological event in the fetal-to-neonatal transition.

The steadily increasing global rate of cesarean sections (CS) has become one of the most debated topics in maternity care. The mode of delivery may have a considerable effect on the state and health of the newborn. CS is a surgical intervention with potential hazards for both mother and child. The opinions of obstetrician-gynecologists regarding normal vaginal delivery (VD) and CS are highly contradictory. The results of previous studies display great differences. We have approached this question from a consideration of oxidative stress and set out to determine a wide range of parameters relating to the oxidative status of neonates born via VD or undergoing CS.

We conclude that the mode of delivery does not have a serious effect on the level of free radical damage if there is no emergency situation. The elective CS does not have an advantage over VD with respect to oxidative stress (Hracsko et al. 2007).

Intrauterine growth retardation (IUGR) is a complication of pregnancy. A newborn with IUGR weighs less than do 90% of all other newborns of the same gestational age. The reported incidence of IUGR ranges between 7 and 10 per cent. This abnormality is associated with increased level of morbidity and mortality, and deformation of the umbilical cord.

The mechanism of development of IUGR has still not been appropriately described, although it is most probably a consequence of an abnormal fetomaternal blood circulation. Accordingly we have carried out examinations on umbilical cord blood and endothelium in order to establish how the antioxidant status of full-term IUGR infants changes and whether the results indicate significant oxidative stress. We compared the antioxidant status and the level of lipid peroxidation (LP) of the umbilical blood in healthy mature neonates and in IUGR neonates. The level of LP was high in the IUGR group while the antioxidant enzyme activities and the levels of antioxidants were significantly

lower in the IUGR group. Damage of proteins and DNA was slightly, but non-significantly higher in the IUGR group. Neonates with IUGR seem to have significant deficiency in antioxidant defense. IUGR is correlated with significant oxidative stress (Hracsco et al 2008).

Nitrogen monoxide (NO) is produced by nitric oxide synthases. The free radical nature of NO and peroxynitrite, renders NO a potent pro-oxidant molecule able to induce oxidative damage and potentially harmful toward cellular targets. Reactive nitrogen species modify amino acid residues, inhibit enzymatic activities, induce lipid peroxidation and deplete cellular antioxidant levels. These features may be associated with the development of different pathologies (Lyll et al. 1996) NO has diverse physiological roles and also known as a vasodilator.

We investigated the NO<sub>2</sub> and peroxynitrite level and the expression of eNOS by RT-PCR in the umbilical cord of IUGR neonates.

Our results support the hypothesis that increased NO production may be a compensatory response to improve blood flow in the umbilical cord. This increased eNOS expression and hence increased NO production in the fetal-placental vasculature may be an adaptive response to the increased resistance pathological pregnancies.

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## Characterization of a family of Arabidopsis receptor-like cytoplasmic kinases (RLCK class VI)

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Arabidopsis possess a large family of receptor-like kinases (RLKs) with more than 600 members (Shiu et al. 2004). Approximately 25% of the Arabidopsis RLKs contain only a kinase domain with no apparent signal sequence or transmembrane region and thus were collectively named as receptor like cytoplasmic kinases (RLCKs). Arabidopsis RLCKs can be subdivided into 10 classes with 193 protein coding genes altogether.

Concerning the function of plant RLCKs, at the present only few members have been characterized and it is very likely that they play major role in the perception and transmission of external signals perceived by RLKs (Zhou et al. 1995; Murase et al. 2004). Moreover, based on our previous investigations and recent literature data, we suppose that kinases belonging to RLCK class VI in Arabidopsis are Rop GTPase targets. Plant specific Rop GTPases are versatile molecular switches in many processes during plant growth, development and responses to the environment and thus a possible implication of RLCKs in these Rop-dependent signal transduction pathways is in discussion.

As part of our investigations related to Rop GTPase-mediated signal transduction in plants, we started to characterize the whole RLCK VI protein family in Arabidopsis. This is underway by studying the genes as well as the encoded proteins. A detailed analysis of the coding sequences and the gene expression pattern of all 14 RLCK\_VI members have already been accomplished. Sequence comparison and phylogenetic analysis revealed that gene duplication played a significant role in the formation of this kinase family and allowed the separation of the 14 RLCK VI kinases into two groups with seven members each (A1 to A7 and B1 to B7). It was established that, several members have an N-terminal UspA ("universal stress protein") domain (group B members) or an N-terminal serine-rich region (group A members) (Jurca et al. 2008).

In order to formulate a possible role of AtRLCK\_VI kinases, real-time quantitative reverse transcription-polymerase reaction (qRT-PCR) was used to determine relative transcript levels in the various organs (root, rosette leaves, cauline leaves, inflorescence stem, flower buds, open flowers, siliques, exponentially dividing cultured cells) of the Arabidopsis plant as well as under a series of abiotic stress/hormone (osmotic, sugar, salt stress, oxidative stress, cold and hormone treatment) treatments in seedlings. The obtained data revealed the differentially regulated expression of the genes, which is in agreement with a high variability of sequence elements in their promoter regions. Thus, the encoded kinase proteins may be involved in a wide variety of signal transduction pathways related to plant development and stress responses (Jurca et al. 2008).

After characterizing the expression of the At-RLCK VI genes, it was imperative to study the proteins itself to find a possible function of these cytoplasmic kinases. Our previous data as well as recent publications indicated that some of the RLCK\_VI members can interact with Rop GTPases. Therefore we decided to establish an RLCK\_VI-to-Rop interaction matrix including 10 members of both families (4 RLCK\_VI and one Rop genes could not be cloned due to various reasons) using the yeast two-hybrid system. As controls, RLCK class IV, VII and IX members as well as alfalfa RLCK\_VI kinases and Rop GTPases were also involved. In general it could be stated that members of RLCK\_VI group A showed interaction with several Rops while that of group B not. The biological role of this interaction needs to be determined. In this direction we further proceed with the in vitro characterization of the activity of these kinases as well as with the produc-

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## Structural analysis of antimicrobial peptides by molecular dynamics methods

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Cationic antimicrobial peptides (AMPs) play an important role in the innate immune system. There are several experimental methods for investigating the secondary structures of these small molecules but they are not precise enough to provide reliable information. Accordingly, we chose molecular dynamics methods to investigate the structural properties of some AMPs. Three types of peptides were studied: peptides rich in His (alloferon-1 and -2), peptides rich in Trp and Arg (indolicidin and tritrypticin) and cyclic peptides containing a disulfide bridge (bactenecin and tigerinin-1).

Alloferon-1 and -2 isolated from insects are rich in His and they possess antiviral and antitumor activities with immunomodulatory effect (Chernysh 2002). The secondary structure of alloferons has not been examined yet. Indolicidin and tritrypticin are peptides containing aromatic residues isolated from bovine neutrophils (Selsted 1992; Lawyer 1996). They possess broad spectrum of antibacterial, antifungal and hemolytic activities. Both indolicidin and tritrypticin are known to be flexible in aqueous solution and adopt either helical (poly-proline II helix) or turn structures in membrane mimic environment. Bactenecin and tigerinin-1 are cyclic peptides with serious antimicrobial activity (Romeo 1988; Sai 2001). Bactenecin was isolated from bovine neutrophils and tigerinin-1 was isolated from the skin of *Rana tigerina*. Each of them tends to adopt  $\beta$ -turn conformation. Because of the controversial assumptions and the lack of reasonable information about the secondary structures of these AMPs our goal was to perform conformational analysis of these peptides.

To explore the conformational spaces of molecules simulated annealing calculations were performed using implicit solvent model. For peptides containing Pro residues, torsional restraints were applied to keep the Xxx-Pro peptide bonds either in *cis* or *trans* configurations. The evolving secondary structures and the intramolecular interactions were examined.

For indolicidin and tritrypticin, it was observed that the *cis-trans* isomerisation plays a key role in the distribution of secondary structural elements (Kerényi 2007). For *trans* isomers, mainly type I and III  $\beta$ -turns were identified. Nevertheless,  $3_{10}$ - and poly-proline II helical segments also appeared along the sequence of peptides possessing *trans* Xxx-Pro peptide bonds. In *cis* isomers, type VI  $\beta$ -turns were observed in specific tetrapeptide units. The stabilizing intramolecular interactions were in good agreement with the structural data: the observed H-bonds play a role in the stabilization of type I and III  $\beta$ -turns, as well as of  $3_{10}$ -helical segments, while the proline-aromatic interactions participate in the stabilization of type VI  $\beta$ -turns. In alloferons, type I, II, II' and III  $\beta$ -turns were the most frequent structural elements. These secondary structures were also stabilized by backbone H-bonds. In the cyclic peptides (bactenecin and tigerinin-1), type I and III  $\beta$ -turns could be found in major population. In the *cis* isomers of tigerinin-1, type VI  $\beta$ -turns were also identified. In every peptide examined, minor populations of backbone-sidechain and sidechain-sidechain H-bonds were also found.

The results obtained from modelling the secondary structures and stabilizing intramolecular interactions were coherent and the conclusions derived from these calculations coincided with the data published so far.

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## Regulation of hox genes in the cyanobacterium *Synechocystis* PCC 6806

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Hydrogenases are widespread amongst prokaryotes, and they play a central role in microbial energy metabolism. The hydrogenase of the cyanobacterium *Synechocystis* PCC 6803, which is a unicellular oxygenic photoautotroph cyanobacterium, is a NiFe-type bidirectional enzyme, that can reversibly oxidize hydrogen (Houchins 1984). However, its physiological role has not been clarified.

Throughout the present investigation, we studied the regulation of the hox genes encoding the bidirectional enzyme on the transcript level by quantitative RT PCR, which was carried out as described elsewhere (Kós PB et al. 2008).

The bidirectional hydrogenase is an oxygen sensitive enzyme (Eisbrenner 1981). Oxygen may affect not only the enzyme activity, but also the expression of the hox genes. In order to verify this hypothesis we studied the effect of anaerobiosis on the hox transcript levels. Lowering the oxygen content of the media below 1  $\mu$ M caused induction of the hox genes.

One hypothesis about the function of the bidirectional hydrogenase is that it plays a role in adapting to new environmental conditions, predominantly adjusting to changes in the intensity and/or spectral quality of light (Appel et al. 2000). According to this idea, it is probable, that the hydrogenase is regulated by photosynthetic electron transport, in particular, by the redox poise of one of the electron carriers of the electron transport chain. We tested if this plausible regulation occurs at the transcript level. Obstruction of the linear electron transport by inhibitors during anaerobic treatment did not alter the induction pattern of hox genes. However, blocking the cyclic electron transport increased the level of the first two genes in the operon, while the last three genes were slightly repressed. These data indicate the existence of a transcriptional regulatory mechanism connected to the cyclic electron transport.

The hydrogenase of *Synechocystis* 6803 is encoded by the hoxEFUYH gene cluster (Bothe H. et al. 1986) which can be transcribed as a single operon (Appel et al. 2005; Oliveira et al. 2005). During anaerobic induction the intensity of the accumulation of the first two genes in the operon (hoxE, and hoxF) differs from the last three genes (hoxU, hoxY and hoxH), implying that there is an additional transcriptional regulatory mechanism acting on the hox operon, which results in an alteration between the transcript levels of the genes within the operon. We supported this assumption by Northern blot analysis.

It has been shown recently that the transcription factor LexA binds to the untranslated region of the hox operon, and suggested to act as a positive regulator of hox gene expression (Appel et al. 2005; Oliveira et al. 2005). During our experiments we monitored the lexA transcript level in parallel with the hox mRNA level. In most of the cases we could not find correlation between the transcript levels of the hox operon, and its putative transcriptional regulator. Furthermore, we frequently observed that changes in their expression levels were opposite to one another. This result shows that lexA is unlikely to act as a direct transcriptional regulator of hox gene expression. Our data is also in agreement with the recent identification of another transcriptional regulator which is also proposed to bind the hox promoter region (Oliveira et al. 2007).

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## The role of nitric oxide (NO), as signalling molecule in root development

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In this work the effects of osmotic stress and exogenous auxin (indole-3-butyric acid, IBA) on root morphology and nitric oxide (NO) generation in roots were compared in pea plants. Five-day old plants were treated with 0, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, 10<sup>-7</sup>, 10<sup>-8</sup> or 10<sup>-9</sup> M IBA or with polyethylene glycol (PEG 6000) at concentrations that determined 0, 50, 100, 200 or 400 mOsm in the medium, during 5 days. NO generation was examined by *in situ* and *in vivo* fluorescence method, using a NO-specific dye, 4,5-diaminofluorescein diacetate (DAF-2DA).

Increasing concentrations of PEG as well as IBA resulted in shortening of primary root (PR), enhancement of lateral root (LR) number and significant increase of NO generation. Time-dependence investigations revealed that in the case of IBA treatments, the LR number increased in parallel with an intensified NO generation, while elongation of PR was not followed by changes in NO levels. Under osmotic stress, the time curve of NO development was distinct compared to that of IBA-treated roots, since significantly, the appearance of lateral initials was preceded by a transient burst of NO. This early phase of NO generation under osmotic stress was clearly distinguishable from that which accompanied LR initiation. It is concluded that osmotic stress and the presence of exogenous auxin resulted in partly similar root architecture but different time courses of NO synthesis. We suppose that the early phase of NO generation may fulfill a role in the osmotic stress-induced signalization process leading to the modification of root morphology (Kolbert et al. 2008a).

As we already know, NO functions in variable physiological and developmental processes in plants (Bartha et al. 2005; Kolbert et al. 2005) however, the source of this signaling molecule in the diverse plant responses is not well understood. Therefore in our further work we provide genetic and pharmacological evidence that the production of NO is associated with the nitrate reductase (NR) enzyme during IBA-induced lateral root development and under osmotic stress conditions (PEG treatments) in *Arabidopsis thaliana* L. NO production was detected in the NR-deficient *nia1*, *nia2* and *Atnoa1* (former *Atnos1*) mutants of *Arabidopsis thaliana*. As inhibitor for nitric oxide synthase (NOS) N<sup>G</sup>-monomethyl-L-arginine (L-NMMA) was applied. Our data clearly show that IBA has increased LR frequency in the wild-type plant and the LR initials emitted intensive NO-dependent fluorescence of the triazol product of NO and DAF-2DA. The presence of increased level of NO was restricted only to the LR initials in contrast to PR sections where it remained at the control level. 200 and 400 mOsm PEG treatments also increased NO fluorescence in roots of *Arabidopsis*. The role of NR in IBA or PEG-induced NO formation in the wild type was shown by the zero effects of the NOS inhibitor L-NMMA. In cases of both treatments the NO synthesis could be inhibited by tungstate treatment, which is a specific inhibitor of NR enzyme. The mutants had different NO levels in their control state (*i.e.* without IBA or PEG treatment), as *nia1*, *nia2* showed lower NO fluorescence than *Atnoa1* or the wild type plant. Finally it was clearly demonstrated that IBA as well as PEG induced NO generation in both the wild type and *Atnoa1* plants, but it totally failed in the NR-deficient mutant. It is concluded that the IBA or osmotic stress-induced NO production is nitrate reductase-associated during lateral root development in *Arabidopsis thaliana* (Kolbert et al. 2008b).

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## Comparative anthropological analysis of non-Hungarian skeletal populations from the 16-17<sup>th</sup> centuries

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The period of the 16<sup>th</sup> to the 17<sup>th</sup> centuries was the age of the Turkish occupation of Hungary. Therefore many Hungarians from the southern region of the country escaped to the north, which was not invaded. On the basis of the archaeological and historical data, it is known that appreciable mass of southern Slav populations immigrated from the Balkan-peninsula and settled down mainly to this deserted, empty, southern countryside (Wicker 2006).

The subject of this research is the comparative anthropological examination of these non-Hungarian skeletal populations from the 16-17<sup>th</sup> centuries. The project has two aims: 1) to describe these populations from an anthropological point of view using osteological age and sex determination, metrical analyses and pathological investigations; 2) to find out the relationship among these non-Hungarian groups and the late medieval Hungarian populations, as well as the origin of the immigrated populations.

The material of this survey is the skeletal population of 6 burial sites (ca. 900 skeletons), which the archaeologists suggest belonged to this immigrated community: Győr-Gabonavásártér, Bácsalmás-Óalmás, Madaras-Bajmoki út, Katymár-Téglagyár, Csávoly-Határ út, Zombor-Repülőtér, (Zombor-Bükkszállás).

To determine the sex and age of death we have used common anthropological methods. The Martin and Saller's (1957) method was applied for measuring the skeletons, and the obtained data have been statistically evaluated with cluster analyses (R Development Core Team 2006). Southern Slav and Romanian series were also involved in the comparison. Paleopathological examinations have been carried out using macromorphological methods, though in certain cases radiographic, histological and molecular biological analyses have been applied as well.

After determination of sex and age, we could establish that in each examined populations the sex ratio was 50% : 50% except Győr-Gabonavásártér series, where the male:female ratio is 70% : 30% – this result might be due to the uncompleted excavation. It is also interesting that in the Bácsalmás-Óalmás series the percentage of infants is high compared to other series, which is due to the well-preserved skeletons and the precise excavation methods.

Many of the skeletons showed different forms of paleopathological lesions, the most common disorders being joint diseases and minor developmental anomalies. According to the prevalence of traumas, the analysed populations could be classified into two groups: a quiet agro-pastoralist population and another group with a more violent lifestyle. Infectious bony lesions were also frequent in each series, many of these cases possibly due to TB-infection (Lovász et al. 2007a). In addition, in the Bácsalmás-Óalmás series 6 cases of scurvy (proved by the histological analyses) were found, a disease rarely described in paleopathological literature (Lovász et al. 2007b). The large number of pathological alterations might indicate a poor state of health in each examined populations.

The results of the statistical analyses indicated that the foreign populations of this study were separated from the late medieval Hungarian series in a distinct group. The comparison of the examined series with the Southern Slav and Romanian data showed that only the southern Bosnian Raška Gora series revealed a close relationship with the foreign ethnic groups in Hungary.

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## Applications of protein and small molecule microarrays

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While DNA microarrays measure changes at the transcription level, protein microarrays can provide information on the protein expression in a parallel way (Tao et al. 2007). Several disease-specific genes and their protein products are overexpressed reflecting the specific genotype of the disease. The direct inhibition of such proteins and the relevant signaling pathways could provide novel opportunities for targeted therapy. Small molecule microarrays (small molecule library printed with high density on a modified glass surface) can be used to screen potential inhibitors of these proteins (Darvas et al. 2004; Walsh et al. 2004).

Protein microarrays manufactured up to date are focused on a specific field (apoptosis, cell cycle, cancer etc.) of the proteome. We tested two different commercially available microarrays for our protein expression studies. We applied the Panorama Ab Microarray Cell Signaling Kit (Sigma-Aldrich) to compare protein extracts from *cerebellum* and *hippocampus* of fat-1 transgenic and wild type (control) mice. The Apoptosis Antibody Microarray (Full Moon BioSystems) was used to investigate the effects of the Ac-177 anticancer compound (from the immunomodulatory drug chemical library of Avidin Ltd.) on apoptosis-related protein expression/phosphorylation.

Small molecule microarrays with 8800 compounds of diverse structures in duplicates were applied for high-throughput screening of protein-ligand interaction studies. A purified serine protease was fluorescently labeled with Cy5 dye and incubated on the microarray. The binding intensity data of each spot representing each compound on the array were determined. To identify its potential inhibitors (molecules which bind to the active site) we incubated the labeled protease with its known substrate on the microarray. Competition for binding between the substrate and the spotted compounds resulted a decreased fluorescence intensity when compared to the substrate-free experiment.

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## The *lemming* gene encodes the Apc11 subunit of the anaphase-promoting complex in *Drosophila melanogaster*

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The ubiquitin-mediated proteolysis of regulatory proteins plays an essential role in regulating the eukaryotic cell cycle. A multi-subunit complex called the anaphase-promoting complex/cyclosome or APC/C plays a key role in this process as an ubiquitin-protein ligase. By targeting mitotic regulatory proteins for degradation, it regulates chromosome segregation and exit from mitosis. The APC/C contains at least 11 subunits, most of which are evolutionarily conserved from yeasts to humans (Castro et al. 2005). The role of most of the subunits within the APC/C complex is still poorly understood.

We have isolated and characterized hypomorph and null alleles of the *lemming* (*lmg*) gene. They show different pupal and pharate-adult lethal phenotypes. Larval neuroblasts from *lmg* mutants show mitotic defects including high mitotic index, chromosome overcondensation, metaphase-like arrest and frequent aneuploid and polyploid cells. Beside the mitotic phenotype, we observed elevated level of apoptosis in *lmg* mutant neuroblasts. Immunostaining of *lmg* mutants shows abnormal cyclin A and cyclin B accumulation in the metaphase arrested mitotic cells.

The *lmg* gene was cloned by plasmid rescue. The predicted coding region consists of 255 nucleotides, and encodes a small, 10 kDa polypeptide containing a RING-finger motif. The Lmg protein shows more than 50% sequence identity and more than 80% sequence homology with the Apc11 subunits of the budding yeast and human APC/C.

Yeast two hybrid experiments revealed that the Lmg protein specifically interacts with a protein identified as the *Drosophila* orthologue of the Apc2/Mr subunit of the yeast and human APC/C (Kashevsky et al. 2002). This interaction was underlined by the synergistic genetic interaction between hypomorph alleles of *lmg* and *mr*.

When introduced and expressed in budding yeast cells, the *lmg* gene was able to fully complement the proliferation defect of yeast temperature sensitive *APC11-myc9* mutant. This result demonstrates that the *lmg* gene product from the fruit fly can functionally replace the yeast APC11 protein.

These phenotypic and functional assays indicate that the *lmg* gene encodes the Apc11 orthologue of the *Drosophila* APC/C. Our work represents the first genetic study of this subunit of the APC/C in a multicellular organism.

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## Network evolution and related models in biology

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Every complex natural system is characterized by several things: redundancy - that ensures information has several good options for circulating across a system, decoupling - the capacity to separate into functional parts that can work even if they are separated, modularity - the property of subparts to work independently and have specific functions, and feedback control - a basic mechanism that allows a system to observe its fitness, making it able to adapt to external or internal pressures. We are investigating how can the properties of general complex systems be measured in evolving networks, and what are the similarities and differences to naturally occurring networks.

Many biological networks evolve using a tradeoff between two basic properties: efficiency, which deals with the capacity of using resources to the maximum extent, and robustness, that deals with resistance to various external pressures. A conceptually simple model of evolution is explained, the outcome of it is however surprising: highly evolved networks have some properties far from many naturally occurring networks.

Simulations of network evolution were done using both a distributed evolutionary algorithm and a random rewiring of the links without the possibility to backtrack in the case of finding a better fitted network, storing the network structures. The efficiency was expressed as computing the number and length of shortest paths, and the robustness by evaluating the efficiency cost of attacking the most central nodes. We used both directed and undirected networks. The resulted over-all topology is showing a highly connected central core surrounded by a dense periphery connected only to the core. Many biological networks have scaled node distributions; this implies that their evolution is never completed, or that it acts modular, in subparts of the network. Several examples are discussed.

The network of protein folding pathways is a particularly interesting one in terms of evolutionary fitness. The number of possible folding states a protein can have during its folding process is huge but the protein is folding extremely quickly, and this is an unsolved problem of today science. Groups of proteins form complexes that generally interact weakly but sometimes the bond can have high specificity. These complexes together can establish a strong structure, but to attain it they need to go through a network of states in a similar manner to protein folding. We are investigating ways to model this in terms of network evolution.

Also not every evolved system is suitable to be expressed as a proper network, but the evolutionary mechanism remains the same. We investigate the fitness of different populations of bacteria forming fractal shaped colonies using an agent based model that simulate the behavior of individual bacteria and the diffusion and sensing of different substances across the medium.

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## Axonal and dendritic effects of neurogliaform cells in rat and human neocortex

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Neurogliaform cells have a unique position among cortical interneurons (Kawaguchi 1995) because they can elicit combined GABAA and GABAB receptor-mediated inhibition on pyramidal cells (Tamas et al. 2003). Moreover, they establish electrical synapses with each other and with other interneuron types (Price et al. 2005; Simon et al. 2005).

We measured the pre- and postsynaptic effects of neurogliaform cells applying simultaneous whole-cell recordings in layers I-IV of rat somatosensory cortex and in human association cortex *in vitro*.

Apart from the GABAA receptor mediated component in postsynaptic responses, single action potentials in neurogliaform cells elicited GABAB receptor mediated responses in neurogliaform, regular spiking and fast spiking interneurons in rat cerebral cortex.

Neurogliaform cells recorded in human cortical brain slices evoked GABAA and GABAB receptor mediated slow inhibition in various types of interneurons and one of them established heterologous electrical coupling. These are the first multiple patch clamp recordings which analyse the functions of neurogliaform cells in human cortex (Oláh et al. 2007).

These cells can effectively recruit GABAB receptors not only on classical postsynaptic compartments like dendritic spines and shafts but on presynaptic axon terminals as well. This presynaptic inhibitory effect can reduce synaptic transmission and this is reflected in the altered paired pulse ratios and reduced amplitudes of the evoked postsynaptic potentials. In one case we show pharmacological dissection of this presynaptic modulation by applying GABAB receptor antagonist.

Our results highlight the peculiar role of neurogliaform cells in cortical circuits and extend their contributions to slow inhibition in cortex.

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## The molecular mechanism of entrainment of the plant circadian clock by light

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At the core of the eukaryotic circadian network, clock genes/proteins form multiple transcriptional/translational negative feed-back loops and generate a basic ~24h oscillation, which provides daily regulation for a wide range of processes. This temporal organization enhances the fitness of the organism only if it corresponds to the natural day/night cycles. Light is the most effective signal in synchronizing the oscillator to environmental cycles. Light signals mediated by photoreceptors are forwarded to the oscillator and cause an acute change in the level/activity of certain clock components that eventually results in a phase shift of the oscillation (Devlin and Kay 2001). Our aim is to reveal the molecular details of this process (also called entrainment or resetting) in *Arabidopsis thaliana*.

The plant circadian oscillator is supposed to consist of three inter-locked feedback loops Locke et al. 2006). In the first loop the morning-expressed CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)/LATE ELONGATED HYPOCOTYL (LHY) transcription factors inhibit the expression of the TIMING OF CAB EXPRESSION 1 (TOC1) gene; conversely, the evening-expressed TOC1 positively regulates the transcription of CCA1/LHY. In the second loop GIGANTEA (GI) induces TOC1 expression during the afternoon/evening, while TOC1 represses GI during the night. Recent data suggested the operation of a third loop, where CCA1/LHY up-regulate the PSEUDO RESPONSE REGULATOR 7/9 (PRR7/9) genes (homologs of TOC1) in the morning and PRR7/9 proteins down-regulate CCA1/LHY expression during the day. In *Arabidopsis*, CCA1/LHY, GI and PRR9 may represent the primary targets of resetting light signals, merely based on the fact that these clock genes are acutely light-inducible. However, the role of their light induction in phase resetting has not been tested directly.

The primary elements of the plant light input pathway are the red/far-red light absorbing phytochromes (PHYA, B, D, E) and blue light absorbing cryptochromes (CRY1,2; Devlin and Kay 2000). However, the molecular links between phytochrome signalling and the core clock components are still missing.

In the first set of experiments we studied the function of the red/far-red absorbing phytochrome B (PHYB) photoreceptor in the resetting process. Our data show that the *phyb-9* mutation affects different parameters (phase and/or period) of rhythmic expression of components of the multi-loop circadian oscillator in *Arabidopsis*. This could be explained by decoupling of the different loops of the oscillator in *phyb-9*. However, we showed that genetic manipulation of a single loop has the same effect on the other loops in wild type and *phyb-9*, which indicates that absence of PHYB does not separate the individual loops. Rather, our data suggest the existence of tissue-specific clocks, which are regulated by PHYB in different ways.

The circadian oscillator responds with characteristic phase shifts to short light pulses. In the second set of experiments we investigated the effect of light induction of certain clock components on the magnitude of such phase shifts. Our data showed that the pattern of light induction of a single clock component does not correspond directly to particular phase responses, but high level of CCA1 and GI induction coincides with strong phase delays, while high level of GI and PRR9 induction coincides with strong phase advances. Phase response curves in single clock mutants indicate that among the light inducible clock components, CCA1, LHY and GI are negative elements of resetting during the subjective night, but PRR9 is a positive element of resetting during the subjective day.

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## Study of the genetic relationships of oribatid mites (Acari, Oribatida) using nucleotide sequences

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Oribatid mites play important role worldwide in soil life, due to their high abundance and soil dwelling lifestyle. They are the most species rich order in the subclass of Acari. Members of different groups of oribatid mites show very different and peculiar morphological appearance, however they belong to single taxonomical unit. There are almost 10 000 described species on the world, from which 523 species are reported in the area of Hungary to date. The species living in Hungary represent 5 major taxonomical units, divided in 77 families and 191 genera. The system of oribatid mites based on morphological features.

In order to study genetic relationships at large and small scale taxonomical levels we chose molecular markers. We used the 160 base pair long part of the 28S D3 nuclear ribosomal DNA coding domain and the 680 base pair long partial sequence of mitochondrial cytochrome-oxidase I (Cox1) subunit. 96 species were collected and determined from different localities of Hungary. 36 species, representing the main

taxonomical groups were selected, and used for the molecular studies. All species used were documented with light and scanning electron microscope images. Distance and likelihood based methods were used in combination with our own and GenBank sequences to determine the genetic similarity between oribatid mites at family level.

Our results suggest that the similarity of 28S ribosomal DNA sequences support the monophyly of lower, but not higher oribatid groups. Cytochrome-oxidase (Cox1) sequences are known to be useful at taxonomical level of genera. We introduced Cox1 sequence in the identification of larva stages.

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## Kynurenines: neuroactive compounds in the central nervous system: An *in vitro* study

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Kynurenic acid (KYNA) is a neuroprotective endogenous tryptophan metabolite produced by astrocytes and neurons via the kynurenine pathway in both humans and rodents. At non-physiological concentrations, KYNA is an excitatory amino acid receptor antagonist that can partially act at both the  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid and N-methyl-D-aspartate subunits of the glutamate receptors (Stone 1993).

In the brain, KYNA is synthesized in astrocytes from its bioprecursor L-kynurenine (KYN) and is then rapidly released into the extracellular compartment. Previous studies have indicated that rat cortical slices have also the ability to synthesize KYNA from exogenously added KYN (Turski et al. 1989). The synthesis of KYNA from KYN is catalysed by kynurenine aminotransferase I and II (KAT I and II) (Schwarcz et al. 2002).

The use of KYNA as a neuroprotective agent is rather restricted, however, because KYNA has only a very limited ability to cross the blood-brain barrier (Fukui et al. 1991). In contrast, KYN and different synthetic KYNA derivatives cross this barrier more readily (Giles et al. 2003).

In the course of the experiments on rat brain slices, the Schaffer collaterals were stimulated and field excitatory postsynaptic potentials (fEPSPs) were recorded in the pyramidal layer of the hippocampal CA1 region. To test the effects of KYNA, we used an *in vitro* pentylenetetrazole (PTZ) model. PTZ, a chemical convulsant frequently utilized in the study of seizures (Yudkoff et al. 2006), exerts its effects by binding to the picrotoxin binding site of the post-synaptic GABA-A receptor (Macdonald et al. 1977). PTZ administered *in vitro* at 1mM induced a considerable increase in the amplitude of the fEPSPs recorded from the hippocampal CA1 region. When applied locally in an extremely high concentration (20 mM), PTZ resulted in characteristic wavelets. However, KYNA administration not only decreased the amplitude of the hippocampal CA1 responses evoked by Schaffer collateral stimulation, but also afforded protection from the PTZ-induced response enhancement.

The KYNA precursor KYN also blocked the development of the PTZ-induced high increase in amplitude. To prove that the KYN→KYNA conversion did take place in our experiments and that it was KYNA which afforded the protection against the effects of PTZ, we applied N-omega-nitro-L-arginine, an inhibitor of KAT I and II (Rózsa et al. 2008).

SZR-72, a synthetic kynurenic acid derivative, applied *in vitro*, proved to be also effective in preventing the high increase in fEPSP amplitudes, generated by PTZ.

These findings show that treatment with KYN, or the synthetic kynurenic acid derivative, SZR-72, even at very low concentration, has an effect on enhanced neural excitability and thus support the hypothesis that manipulations of the kynurenine pathway might be a rewarding target in different neuronal disorders affected by neuronal hyperexcitation.

Additionally we have shown, that KYNA in submicromolar concentration range has a positive neuromodulatory effect. In nM concentrations, kynurenic acid does not give rise to inhibition, but in fact facilitates the field excitatory postsynaptic potentials, recorded from the hippocampal CA1 region (Rózsa et al. 2008).

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## Examination of the hydrogen-metabolism in *Methylococcus capsulatus* (Bath)

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*Methylococcus capsulatus* (Bath) is a Gram-negative, methylotrophic bacterium, which oxidizes methane to carbon dioxide for energy generation. The enzyme complexes methane monooxygenases (MMOs) oxidize methane to methanol and co-oxidize a wide variety of aliphatic, aromatic and halogenated hydrocarbons, therefore they are extremely versatile enzymes for biocatalysis and bioremediation. The *in vivo* electron donor of the MMOs is NADH, which must be regenerated. Since biodegradation processes using MMO are co-oxidation processes, alternative ways of supplying reducing power are needed. Possible candidate could be H<sub>2</sub> for NADH + H<sup>+</sup> generation.

Hydrogenases are metalloenzymes catalyzing the reversible oxidation of H<sub>2</sub>. *M. capsulatus* (Bath) contains a soluble (Hox) hydrogenase - which is able to reduce NAD<sup>+</sup> using H<sub>2</sub> - , and a membrane-bound nickel-iron Hup hydrogenase - which plays an important role in the recycling of hydrogen, and maybe donates the electrons to the quinone pool. Another enzyme - nitrogenase - produces H<sub>2</sub> as a byproduct under nitrogen fixing condition.

$\Delta hupSL$  and  $\Delta hoxH$  deletion mutants were generated (Csáki et al. 2001). H<sub>2</sub>-driven MMO activities of these mutants and wild type were measured to obtain information about the *in vivo* function of the hydrogenases (Hanczár et al. 2002). The deletion mutants revealed unexpected behavior: the  $\Delta hupSL$  mutant did not show H<sub>2</sub>-driven MMO activity, while the  $\Delta hoxH$  mutant showed. The Hup hydrogenase - which is unable to reduce NAD<sup>+</sup> directly - is required for the H<sub>2</sub>-driven activity of MMO. To understand the role of Hup hydrogenase in H<sub>2</sub>-metabolism the first step is to find all genes coding for proteins, which has any effect on Hup hydrogenase activity.

Several Hup<sup>-</sup> phenotype mutants were isolated from a *M. capsulatus* random mutant library, which was generated by transposon mutagenesis. The transposon was found in a structural gene (*hupL*), in an accessory gene (*hupD*) of Hup hydrogenase, and in other genes: TonB-dependent receptor-like putative protein coding gene (*tonB*) and conserved hypothetical protein for NADH ubiquinone/plastoquinone complex coding gene (*nupX*).

The *in vivo* H<sub>2</sub> production capacities of the wild type and the mutant strains were compared. The Hup hydrogenase of the wild type consumed a lot of H<sub>2</sub> from the gas phase, while the mutants had lower H<sub>2</sub> consumption activity both under nitrogen fixing and nitrogenase repressed conditions. Hup hydrogenase structural proteins were detected both in wild type, HupD<sup>-</sup>, TonB<sup>-</sup> and NupX<sup>-</sup> transposon mutants with HupL antibody by Western Blot assay, in contrast to the  $\Delta hupSL$  deletion and HupL<sup>-</sup> transposon mutants.

The results show the presence of the matured Hup hydrogenase in TonB<sup>-</sup> and NupX<sup>-</sup> transposon mutants, but hydrogen-metabolism of these mutants is damaged, therefore they have Hup<sup>-</sup> phenotype. According to the *in silico* analysis and global protein alignment the proteins of the *nupX* containing operon are similar to the NuoM, NuoL and NuoN proteins of the NUO (NADH ubiquinone-plastoquinone oxidoreductase) complex. In our hypothesis the proteins of the examined nuo-like operon maybe play a role in the energy conversion of the bacterium, while the examined TonB-dependent receptor-like putative protein perhaps takes part in the mechanism of TonB-catalyzed iron transport through the bacterial cell envelope, indirectly contributing to the assembly of the membrane-bound nickel-iron Hup hydrogenase.

To determine the role of the mutant genes further investigations are needed.

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## Macro-organization and structural flexibility of the light-harvesting system of diatoms (Bacillariophyceae) and their significance in the photosynthetic light energy utilisation

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Among the phytoplankton species of marine and freshwater communities, diatoms play a dominant role in the biogeochemical cycles of carbon, nitrogen, phosphorus and silicon with a strong impact on the global climate. Since diatoms experience randomly fluctuating light intensities and large scale temperature changes, they have developed various mechanisms of photoprotection.

In higher plants, it has been established that the photosystems (PSs) with their peripheral chlorophyll *a/b* light-harvesting antenna complexes (LHCs) form supercomplexes. The PSII-LHCII and PSI-LHCI supercomplexes are laterally segregated in the granal and stromal thylakoid membranes, respectively (cf. e.g. Mustárdy and Garab 2003). It has also been shown that LHCII and PSII-LHCII are assembled into macrodomains with long-range chiral order, which possess remarkable structural flexibility and by this means the structural flexibility of the macroassemblies plays an important role in the regulation of the light energy conversion (Garab 1996).

Diatoms contain specialized peripheral light-harvesting antennas, the fucoxanthin-chlorophyll *a/c* proteins (FCPs), instead of LHCs. FCPs are also intrinsic light-harvesting complexes but their carotenoid is the fucoxanthin and contains chlorophyll *c* as accessory pigment. Compared to higher plants, our knowledge concerning the arrangement and the supramolecular organization of the antenna complexes in the thylakoid membranes is quite rudimentary, and much less is known about their possible role in different regulatory processes.

The major aim of our studies was to characterize the (macro-)organization of the complexes in *Phaeodactylum tricorutum* and *Cyclotella meneghiniana* cells, as well as on isolated thylakoid membranes and FCPs. By using circular dichroism (CD) spectroscopy, we found that the spectra of the whole cells were dominated by an intense band at (+)698 nm, with typical psi-type features (psi, polymerization or salt-induced). This band, which appeared to be associated with the multilamellar membrane architecture, was sensitive to the light intensity during growth, to the osmotic pressure of the medium and to heat. We also found that it was capable of undergoing reversible changes upon illumination with actinic light. In isolated thylakoid membranes, the psi-type CD band, which was lost during the isolation procedure, could be partially restored by addition of Mg<sup>2+</sup> ions; the same treatment was also important for optimizing the quantum yield of PSII and the non-photochemical quenching of chlorophyll *a* (Szabó et al. 2008). With a refined isolation method, we were able to isolate the oligomeric form of FCP, which represented the native form of the antenna system in thylakoid membranes of diatoms (Lepetit et al. 2007). We also gained information on the orientation and local environment of a special fucoxanthin pigment molecule of the FCP, which exhibited an extremely strong electrochromic response and intense linear dichroism (LD) signal at around 550 nm, most probably given rise by strong fucoxanthin/chlorophyll *c* interaction.

In summary, our data have shown the presence of highly flexible macroassemblies of the light-harvesting system in diatoms, which also appears to participate in different regulatory processes of the photosynthetic light energy conversion.

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## Characterization of beta-glucosidase enzymes and their coding genes from the fungal class Zygomycetes

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The genus *Rhizomucor* (Zygomycetes, Mucorales) comprises two well-established thermophilic species, *R. pusillus* and *R. miehei* (Vágvölgyi et al. 1999). Both of them are well known from biotechnological applications in consequence of their effective extracellular enzymes, e.g. proteases and lipases (Rao et al. 1998). Beta-glucosidases play important roles in biology, including the degradation of cellulose biomass by fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants (Bhatia et al. 2002).

Filamentous fungi are known to be good producers of beta-glucosidases and several fungal glucosidases have been isolated and analyzed. Unfortunately, Zygomycetes are poorly characterized from this aspect. In the frame of a recent study, beta-glucosidase activity of

several Zygomycetes fungi was tested in solid-state fermentation assays. Some *R. miehei* strains showed intensive extracellular enzyme activity. The aim of our present study is the identification and molecular and biochemical characterization of a beta-glucosidase enzyme and its coding gene (*bgl*) from *R. miehei*.

Degenerated beta-glucosidase-specific primer pairs were designed to conserved regions of fungal glycoside hydrolase family 3 genes and a 493 bps long fragment was amplified by PCR from the genomic DNA of the *R. miehei*. The sequence of the amplicon was determined; it showed high homology with the C-terminal domains of the beta-glucosidases belonging to the family 3. Based on this sequence, specific primers were designed for inverse PCR. The original fragment has been lengthened to a 4063 bps long sequence which contained the 2826 bps long beta-glucosidase gene encoding a protein with a length of 743 amino acids. *Rhizomucor bgl* showed the highest homology with the beta-glucosidases of *Phanerochaete chrysosporium*, *Trichoderma reesei* and *Piromyces* sp. strain E2.

For gene expression studies two transformation vectors were constructed: the plasmid pTM1 contained *bgl* under the control of the regulator sequences of the related *Mucor circinelloides gpd1* gene, while the plasmid pTM4 harboured the promoter region of the *bgl* fused with a green fluorescent protein gene. In the lack of an efficient transformation system in *R. miehei*, genetic transformations were started in a heterologous system: PEG-mediated protoplast transformations were performed in an uracile auxotrophic *M. circinelloides* strain. Induction of the *bgl* promoter by different substrates was studied in the *M. circinelloides* transformants harbouring the pTM4 plasmid. Strong fluorescence was observed only in the transformants growing on cellobiose containing medium. Analysis of the transformants containing pTM1 is in progress.

For production of the extracellular beta-glucosidase enzyme in high amount, *R. miehei* was grown on wheat bran medium for six days at 40°C. The enzyme was purified from the crude extract to homogeneity by ammonium sulphate fractionation and two-step chromatographic separation through Sephadex G100 and G200 columns was performed. The molecular mass of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis. The optimum temperature and pH for the action of the enzyme were at 60°C and 4.0 to 5.0, respectively; the beta-glucosidase proved to be highly stable at temperatures up to 50°C but it almost lost its activity at temperatures above 70°C. The enzyme was fairly stable at pH 4.0 to 6.0 and 20% of the activity remained after incubation at pH 3.0.

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## Map based cloning of leaf developmental abnormality in *Medicago sativa* and comparison of the rDNA (NOR) regions in *Medicago truncatula* and *Medicago sativa*

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A *Medicago sativa* mutation called sticky leaf (*stl*), which appears in the nature occasionally was reported previously (Stanford 1959) and used as a morphological marker in cross-fertilizations. The *stl* mutant is characterized by the adhesion of the adaxial sides of adjacent leaflets in the same leaf, as well as adhesion of opposite halves of the same leaflet. The inheritance of the mutant in tetraploid populations suggested that the *stl* character is determined by a single recessive gene (Stanford 1965). This was confirmed later in a diploid F2 segregating population originating from a cross between *M. sativa* ssp. *quasifalcata* and *M. sativa* ssp. *coerulea* (Endre G. PhD thesis 1997). Genetic mapping of this trait in this population placed the *Stl* gene on Linkage Group six (LG6) in the close vicinity of the rRNA coding region (NOR). In the closely related model legume *M. truncatula* that shows very high overall macrosynteny with *M. sativa* the position of NOR region is different, it is located on LG5.

Our aim is to identify the mutant gene which is responsible for the *stl* phenotype in *M. sativa* with the help of the available genomic information of the model *M. truncatula*. For this the localization of the ortholog *Stl* gene in *M. truncatula* is needed, therefore we compare the NOR and its flanking regions in these *Medicago* species. To explore this syntenic relationship we used three approaches. One of these was to map the molecular markers linked to the *M. sativa stl* trait on the *M. truncatula* linkage map. The second direction was to search for and use structural genomic information of *M. truncatula* sequenced BAC clones carrying rDNA sequences. We have identified the repeat unit sequences and looked for discrete sequences for mapping on *M. sativa*. As a third approach we have screened a *M. truncatula Tnt1* insertional mutant plant collection for leaf phenotypes similar to *stl*.

Molecular markers Q5C and P16C closely linked to *stl* phenotype on the *M. sativa* map were used to identify *M. truncatula* BAC clones. Some have already been mapped in *M. truncatula*, but their position was neither on LG5 near NOR region nor on LG6 in syntenic position of *stl* in *M. sativa*. We have subcloned and sequenced other BACs with unknown location but only intergenic repetitive sequences were identified not suitable for syntenic mapping purposes.

Following the second approach with the help of BAC sequences we determined the rDNA units (the 18S-5.8S-25S rRNA coding + IGS sequences) in *M. truncatula*. The thorough analyses of these BACs identified only one putative other coding sequence but so far mapping efforts failed in *M. sativa*.

In the meantime a publication reported about positioning *M. truncatula* FUT2 ( $\alpha$ -1,3-fucosyltransferase) genes by FISH method on five chromosomes (LG1,4,5,7,8), one of them inserted in the NOR region. Based on this information we checked if FUT2 gene was present also in *M. sativa* NOR region or not. Southern blot experiments suggested that FUT2 gene has lower copy number in the *M. sativa* genome and no position inside the rDNA region was detected. This further suggests a low synteny between these regions in the two *Medicago* species.

Insertional mutagenesis technology is important tool for isolation of new genes by phenotypes (forward genetics) or study their function (reverse genetics). We have found a number of plants with leaf phenotype similar to *stl* among *M. truncatula* insertional mutants carrying tobacco *Tnt1* retrotransposons. Sequence of *Tnt1* flanking regions of these mutant lines were determined by AFLP-PCR method. These sequences have been analyzed by their potential coding function as well as by their map position. Possible candidates were identified based on location (Mt LG5, LG6 or unknown) and are subject for further studies.

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## Typing of bacterial symbionts of entomopathogenic nematodes, and their potential use as biocontrol agents

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The extensively used biocontrol organisms, entomopathogenic nematodes belong to the *Heterorhabditis* and *Steinernema* genus are symbiotically associated with *Photorhabdus* and *Xenorhabdus* bacteria. The bacterial partners have an outstanding role in the life-cycle of their nematode hosts: they produce wide range of toxins, hydrolytic exoenzymes and antibacterial compounds that are responsible for the death and bioconversion of the infected insect larvae and prevent other soil organisms from degrading the insect cadavers. The bacterial partners highly determine the effectiveness of the symbiotic complex against different insects, therefore bacteria have an interests from the viewpoint of biocontrol practice. The aim of this study was to survey the diversity of the Hungarian *Photorhabdus* isolates, and to obtain comprehensive view about their potential use as industrial entomotoxin and antimicrobial compound producers.

*Photorhabdus* strains from entomopathogenic nematodes isolated from Hungarian soils (Tóth 2006) were characterized by morphological, physiological and genetic properties to survey the diversity of bacterial symbionts of *Heterorhabditis* species of commercial importance. Entomopathogenic bacteria (EPB) were isolated from 245 entomopathogenic nematode strains originated from different part of Hungary. There were 156 *Photorhabdus* and 77 *Xenorhabdus* from the successfully cultured 233 EPB isolates. 65 *Photorhabdus* isolates representing the whole collection from the point of view of geographical and nematode host distribution were analysed. First stage bacteria cells selected on NBTA indicator plates were used to determine the morphological traits and to perform physiological tests using Biolog GN microplates and API20E strips. Cytotoxic and antibacterial properties of cell-free culture broth were measured against *Drosophila melanogaster* S2 and *Spodoptera frugiperda* Sf9 cell lines or *Staphylococcus aureus* and *Bacillus subtilis* bacteria, respectively. Morphologically and physiologically homogenous groups of *Photorhabdus* isolates were characterized by partial sequencing of 16S rRNA and *gyrB* subunit gene.

High physiological and morphological diversity were proved among the *Photorhabdus* isolates, and all of physiological and morphological bacteria types could be isolated both from *Heterorhabditis megidis* and *H. downesi*. A number of bacteria isolates were shown only moderate 16S rRNA gene sequence similarities with type strains of all described *Photorhabdus* species/subspecies. Using *gyrB* sequences to the phylogenetic analysis, these isolates were proved to be part of the species *Photorhabdus temperata*, with clear separation from both palearctic and American strains (phylogenetic distances are 93.1% and 92.1%, respectively). The physiological and carbon source utilization characters supported the phylogenetic position of these strains, therefore a new subspecies, *Photorhabdus temperata* subsp. *cinerea* (Tóth and Lakatos 2008).

The 39% and 13% of all studied isolates were ineffective against *S. aureus* and *B. subtilis*, respectively, while 26% and 7% were much more effective, than 100 ppm streptomycine, which was the control. About 10% of the studied isolates do not produce effective ingredients against *S. aureus* and *B. subtilis* bacteria, while 10% of them were highly effective against both bacteria.

59% and 8% of isolates had no cytotoxic effect on S2 and Sf9 cells, while 3% and 21% were highly toxic to dipteran and lepidopteran cells. There was not any *Photorhabdus* isolates, of which fermentation liquid was toxic to both cell types.

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## Engineering plant abiotic stress tolerance by the overexpression of aldo/keto reductases

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Due to the sessile life style, plants are continuously exposed to a wide range of biotic and abiotic stress factors. This stress exposure severely affects their bioproductivity by causing the rapid and excessive accumulation of reactive oxygen species (ROS). ROS production in the vicinity of biomembranes containing polyunsaturated fatty acids can lead to lipid peroxidation and generate chemically reactive cleavage products, largely represented by aldehydes. Plant aldo/keto reductases (AKRs), among other enzymes, have been shown to be effective in the detoxification of lipid peroxidation-derived reactive aldehydes (Oberschall et al. 2000; Hideg et al. 2003).

In the present work we characterize a novel rice (*Oryza sativa*) AKR protein (OsAKR1) and investigate the transcriptomic changes in the gene expression profile of additional two AKR genes (*OsAKR2*, *OsAKR3*) in response to different stress treatments. A wide range of stress factors (abscisic acid, hydrogen-peroxide, mannitol etc.) was shown to trigger the expression of these AKR genes in rice cell suspensions, resulting in several folds of increased transcript levels. The most effective inducers were the ABA and hydrogen-peroxide, and *OsAKR1* gene turned out to be the most stress responsive. Stimulated by these results we investigated further the properties of the encoded protein by the *OsAKR1* gene, by cloning the full-length *OsAKR1* cDNA into recombinant protein expression construct, and purifying the glutathione-S-transferase (GST)-OsAKR1 fusion protein. Results of subsequent assays revealed that the GST-OsAKR1 recombinant protein exhibited a high, NADPH-dependent catalytic activity to metabolize toxic aldehydes (methylglyoxal, phenylglyoxal, glyoxal). Since cytotoxic reactive aldehydes can produce significant damages in the plant cells, the function of OsAKR1 protein to metabolize some of these harmful products was very promising. We also showed through *in vivo* experiments, that overproduction of this enzyme in *E. coli*, increased the tolerance of bacterial cells against high concentration (2mM) of methylglyoxal. The stress induced transcription of this AKR gene, as well as the data obtained from its biochemical characterization, supported its possible involvement in the abiotic stress induced reactive carbonyl detoxification pathways.

Till now there are several approaches to increase stress tolerance by manipulating the expression of endogenous, stress-related genes. Strategies targeting transcription factor expression have been shown to be effective, but on the other hand, stress tolerance can also be achieved by changing the expression of a single gene (Zhu 2001). Following the latter approach, we overexpressed the *OsAKR1* gene in tobacco (*Nicotiana tabacum*) and verified the effects of a single gene overexpression on the stress tolerance of the transgenic plants. We found, that the transgenic lines overproducing the OsAKR1 protein, accumulated significantly lower reactive aldehydes in response to the methylviologen (MV) treatment than the wild type. MV is a strong oxidative stress inducing herbicide, linked to ROS production and consequently to the formation of toxic aldehyde degradation products. In addition, the overexpressing lines reserved their photosynthetic functions more efficiently after heat treatments than the wild type. Therefore we suggest, that overexpression of a single gene (*OsAKR1*) and the accumulation of OsAKR1 protein is mainly beneficial in the detoxification processes against the reactive aldehydes generated at increased levels under stress conditions in the transgenic plants.

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## Toxicogenomics screening of small molecules using high-density nanocapillary QRT-PCR technique

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Toxicogenomics combines studies of genomics, cell and tissue-wide protein expression and metabonomics to understand the role of gene-environment interactions in healthy and diseased samples. Predictive toxicogenomics is the acquisition of advanced knowledge of the safety profile of a compound using genomic biomarkers (Fielden et al. 2006). By clustering analysis of the gene expression profiles over selected biomarkers induced by the lead molecules and relevant derivatives, the medicinal chemist can deduce the relationship between structural modifications and changes in the toxicity profile (structure-toxicity relationship). Involvement of well-characterized reference compounds can be of help in this profiling, for instance defining the specific tissue or organ toxicity.

Using in-house validated chemical reactions that are suitable for parallel synthesis and a collection of multifunctional „drug-like” scaffolds, a dedicated discovery screening library of 10,000 compounds has been enumerated by a cascading diversity building approach ([www.amrigo.com](http://www.amrigo.com)). Based on the cytotoxicity measured in MRC-5 human fibroblast assay, further on HepG2 human hepatocarcinoma assay and the interpolated  $IC_{50}$  values, 668 compounds were selected aiming for maximal diversity of scaffolds.

These selected 668 small, drug-like compounds of unknown effects and other, toxic compounds of known and of yet unknown effects and pharmaceutical active entities were screened for their gene expression profiles *in vitro*, over 56 selected biomarkers (toxicology, transporters). Our objective was to see to what extent the highly similar chemical structures induce similarities in their hepatotoxic fingerprints and to test the analytical performance of the nanocapillary QRT-PCR technique and its general applicability for the field of toxicogenomics.

Preliminary tests have been performed with our inhouse ToxicoScreen DNA-microarrays (Vass et al. 2006) and with the traditional QRT-PCR technique, following which we shifted to the OpenArray nanocapillary quantitative real-time PCR-technology (Morrison et al. 2006; Avidin Ltd.-BioTrove Inc.) that has meanwhile appeared on the market. This later technology merges the high-throughput of DNA-microarrays with the sound characteristics of QRT-PCR.

By the combination of a relatively large combinatorial chemical library and a relatively small set of selected toxicological biomarkers, we intended to avoid the two culprits of toxicogenomics: ‘the curse of dimensionality’ (too many genes), and ‘the curse of dataset sparsity’ (too few samples). The generally accepted, however rarely adapted sample-per-feature ratio for robust clustering performance is at least 5 to 10.

Based on the scaffold structure or the characteristic residues, we assigned the tested chemicals into subgroups. Different clustering methods were applied, based on results from unsupervised hierarchical clustering we performed supervised, K-means clustering. Our objective was to see whether the correlation between gene expression fingerprint and structure of the compound inducing it can be detected and to what extent can this correlation be rooted back to the scaffolds.

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## Tools for improving stress adaptation in cereals

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Abiotic stresses are very important factors that reduce crop productivity. Plant root is the primary organ for uptake of water and nutrients, therefore it plays important role in tolerance to stresses like drought or salinity. Plants developing stronger and deeper roots suffer less from water deficit. The aim of our work was to improve the stress-tolerance in cereals using transcriptome analysis of rice cultivars under drought stress conditions, and stress-induced and root-specific promoters.

Genes facilitating development of efficient root system can increase the survival of the plant. Fusions of drought stress-related root-specific promoters to these genes may provide environment friendly and efficient solution to improve roots of crop plants under stress conditions.

Based on published data two candidate promoters were selected: the rice *CatB* and the *RSOsPR10* promoters. The *CatB* promoter is known to be root-specific (Iwamoto et al. 2004), the expression of the *RSOsPR10* mRNA is high in salt and drought stress conditions in root (Hashimoto et al. 2004). Both promoters have stress-related transcription factor binding sites and (MYB, WRKY, DREB, LTRE) in their sequence. The 1.6 kb *CatB* promoter and the 2 kb of the 5' flanking region of the *RSOsPR10* were cloned, and fused to reporter genes. The constructs were transformed into rice calli and tobacco leaves.

On the regenerated  $T_0$  rice plants, salt stress was performed that revealed the *RSOsPR10* promoter directing root-specific and stress-induced expression pattern of GFP reporter. *CatB::GUS* transformed  $T_0$  and  $T_1$  tobacco plants showed root and vascular bundle-specific GUS expression, and induction under salt stress.

The changes of the rice root transcriptome under stress conditions and its alterations during a daytime period were investigated in a greenhouse experiment including three cultivars growing in a sand-perlite soil mixture. The stressed plants were irrigated with 20% of water for one month, causing drought-stress condition. The samples were collected three times in a day from each genotype both from drought stressed and control.

To follow the transcriptional changes, root samples from the most tolerant genotype were hybridized with rice oligonucleotide DNA chip. 3200 of the genes represented on the chip gave signal in all of the hybridizations, and 11.6% were up-regulated, and 6.7% were down-regulated in the adaptive cultivar.

Based on the expression profiles of genes during the day under drought-stress, eight clusters were built, and functional categorization was done based on the known or putative function of the encoded proteins, following the classification established by Yang et al. (2004).

Comparing the ratios of the gene-classes between the induced and repressed genes four groups showed significant difference in favor of the up-regulated genes. The ratio of the genes encoding proteins involved in metabolism, signal transduction and cell growth was higher among the induced genes.

To validate the results of the chip-hybridization, and to find stress-induced and root-specific genes, quantitative real-time PCR experiments were performed. Seven genes were tested, and the chip-hybridization results were confirmed for four of them: an *ABA/WDS induced* gene, a *LEA group 3*, a putative *LEA*, and a gene with unknown function. Furthermore, these genes' expression levels were determined in the shoot samples in all of the three genotypes. The alterations of the expression patterns reflected the differences in the stress-tolerance of the three cultivars. However all of them showed higher induction in roots than in shoots, the *LEA group 3* gene appeared to be the most stress-inducible and root-specific, becoming a candidate to develop expression cassettes including this gene.

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## The study of the Nimrod protein and gene cluster in *Drosophila melanogaster*

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Every multicellular organism has to maintain its homeostasis in masses of invading microbes in its environment. The innate immunity constitutes the first line of defence against this challenge. As the defence mechanism of *Drosophila melanogaster* consists of innate immune processes only, lacking an adaptive immune system, it can be characterised as a less complex homologue of its mammalian counterpart, making the fruit fly a valuable model organism for studying innate immune responses.

The circulating blood cells of the fruit fly are key effectors of its immune defence. These hemocytes can be divided into three characteristic types. In wild type third instar larvae 97% of all circulating hemocytes are plasmatocytes, but even though it is the most abundant cell type in circulation, previously it could only be characterised based on its phagocytic capacity.

Our group identified the first plasmatocyte-specific immunological marker. After screening thousands of candidate hybridoma clones, we found two specific monoclonal antibodies, recognising different epitopes on the same molecule. We purified the antigenic protein, and analysed it with MALDI-TOF. A protein was identified with characteristic domain structure, containing a signal peptide, a CCxGY motif, EGF repeats, one transmembrane and an intracellular domain.

We have shown that the protein functions as a putative phagocytosis receptor of the plasmatocytes. As this protein helps the major sentinel cells catching bacteria we decided to name it after the big hunter Nimrod (*nimC1*; Kurucz et al. 2007b). The RNAi induced loss of function *nimC1* mutant plasmatocytes phagocytose *S. aureus* bacteria at significantly lower levels than the wild type, but *E. coli* phagocytic capacity was not compromised. On the other hand ectopic expression of the *nimC1* gene in S2 *Drosophila* cell line significantly enhanced the phagocytic capacity of cultured cells.

As it seemed that NimC1 is not the only phagocytosis receptor in *Drosophila*, we did in silico analysis searching for similar proteins in the fruit fly genome. Nimrod belongs to the superfamily of EGF repeat containing proteins, from which only 12 contain the characteristic CCxGY motif. In these proteins one or more EGF domains can be found, which fit a specific, more stringent consensus sequence, which we named NIM repeat. Nine of these genes, including *nimC1* can be found in a genomic region spanning 88 kilobase on the second chromosome. (Kurucz et al. 2007a; Somogyi et al.)

To prove, that these predicted genes are really transcribed, and to characterise their expression pattern we performed reverse transcription polymerase chain reactions using samples from whole third instar larvae, isolated hemocytes in wild type and hemocyte overproducing *l(3)mbn-1* mutant, and wild type adults.

All of the predicted genes were transcribed in every studied condition; the only exception was *nimA* which did not show any transcription activity in hemocytes.

In order to study the expression pattern of the *nimC1* homologues in more detail, we intend to produce new specific antibodies recognising the NimC1 homologue proteins. In order to acquire suitable amount of isolated protein for immunization, we have cloned the non-homologues regions of four different *nim* genes into prokaryotic expression vectors containing a HIS affinity tag, allowing isolation of the produced protein. In order to allow possible post-translational modifications of the expressed proteins, two of them are already cloned into affinity tagged eukaryotic expression vectors.

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