Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARIES

In vitro analysis of the regulation of the human DNA damage tolerance pathway

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The DNA in our cells is continuously damaged by different agents, such as UV irradiation, reactive oxygen species, metabolites and chemicals. These agents are changing the structure of the DNA molecule, leading to mutations during their replication. To avoid these mutations many DNA repair mechanisms have evolved. These mechanisms are able to set back the original structure of the DNA double helix but some damages get to the S phase of the cell cycle where they can cause the stalling of the replication fork, chromosomal breaks and cell death. To avoid these possibilities the DNA damage bypass pathway has evolved which can protect the stalled replication fork by different ways.

The main step of the pathway is the monoubiquitylation of the PCNA protein, which is the processivity factor of the polymerases by Rad6/Rad18 complex at the lysine 164 position. After this modification the replicative polymerase can be changed by an alternative polymerase, which is able to synthesize through the lesion. In an other error free mechanism the monoubiquitylated PCNA becomes polyubiquitylated by the Mms2/Ubc13/HLTF complex through lysine 63 residues, which can facilitate HLTF dependent replication fork reversal. On this newly emergent so-called chicken foot structure the stalled replication can be rescued using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism.

Our study is focusing on the better understanding of the function and regulation of the DNA damage bypass pathway. A stalled replication fork is surrounded by various DNA-binding proteins which can inhibit the access of damage bypass players, and it is unknown how these proteins become displaced. We found that HLTF has an ATP hydrolysis-dependent protein remodeling activity, by which it can remove proteins bound to the replication fork. Our ultimate goal is to shed light on the whole molecular mechanism of the damage bypass.

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The consequence of PrP^C or Shadoo overexpression on the cytotoxic effect of a ΔCR-PrP mutant phenotype

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Transmissible spongiform encephalopathies (TSE) are lethal neurodegenerative disorders with characteristic spongiform degenerations of the brain and variable degree of amyloidal plaque formation. TSE is caused by the conformational transition of the cellular prion protein (PrP^C) to an abnormal isoform, commonly referred as PrP^{Sc}. PrP^C is a glycoprotein on the cell surface, anchored to the outer leaflet of the plasma membrane by a glycosylphosphatidyl-inositol (GPI) moiety. PrP^C expressed ubiquitously in the body reaching highest levels in the central nervous system and the heart. In TSE neurotoxicity is coupled to transmissibility.

However, with deletion mutant PrPs that are expressed in PrP knockout mice, the toxicity and the transmissibility can be separated: (i) Upon infection of transgenic mice expressing GPI-anchorless PrP, PrP^{Sc} is formed but the mice did not show any sign of neurodegeneration. Thus, the formation of GPI-anchorless PrP^{Sc} is not neurotoxic, although anchorless PrP^{Sc} can cause neurodegeneration in wild type mice when transmitted into their brains. (ii) In case of internal deletion PrP mutants, the expression of the mutant proteins is neurotoxic, the mice develop a neurodegenerative phenotype that is reminiscence of that seen in TSE, but this prion disease is not transmissible and the deletion mutant proteins do not convert to an abnormal conformation. Understanding the mechanism of the neurotoxic effect of the internal deletion mutant prion proteins might help to understand the mechanism of the neurotoxicity of the conformational conversion of PrP^{Sc}. Additional advantage of this model system is that no infectious agent involved, that simplify several steps in the research project.

The deletion mutant of the prion protein missing the segment, called Central Region, referred as ΔCR mutant, causes a neonatal lethal phenotype when expressed in PrP knockout mice, provides an alternative approach in the understanding of the physiological function of PrP^C and how PrP^C can be degraded to produce neurotoxic effects. Expressing ΔCR-PrP, in mammalian neural cells has been shown to cause hypersensitivity to the toxic effects of antibiotics using for stable cell line selection. This hypersensitive phenotype can be rescued by co-expression of wild type PrP^C.

Our aim is to establish a model system for studying the toxic effect of ΔCR-PrP in mouse neuronal N2a and human neuronal SH-SY5Y cells, for examining whether Shadoo (Sho, a member of the Prion protein family) like wild type PrP can also rescue this phenotype and for discerning the underlying mechanism.

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We established stable cell lines, which expressed the ΔCR-PrP mutant or wt PrP^C with the reporter gene GFP. Subsequently, in order to achieve a high overexpression of wt PrP^C or Sho in the ΔCR-PrP expressing cells lentiviral transduction is used. For assessing the drug hypersensitivity caused by the expression of ΔCR-PrP, the cell viability with or without Zeocin treatment is measured using an MTT assay.

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A comprehensive view of the determinants of molecular evolution in yeast

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Why do genes evolve at different rates? It is a well-known phenomenon that amino acid sites of protein sequences undergo substitutions during the course of evolution, and the rate of this change varies widely across genes. In the past years the major determinants underlying protein sequence evolution have been largely uncovered. However, molecular evolution is not restricted to amino acid substitutions, but rather encompasses various aspects of molecular changes from the deletion and duplication of whole genes to change in expression levels and subcellular localizations. Importantly, while the rate of sequence and expression divergence has been thoroughly studied, gene duplicability and the propensity for gene loss remain poorly understood, and it remains completely unexplored what determines the propensity for change in subcellular localization.

Here we aim to systematically explore and compare the genomic and functional genomic properties determining i) sequence divergence, ii) gene expression divergence, iii) propensity for gene loss, iv) gene duplicability and v) propensity for change in subcellular localization of proteins.

We compiled a dataset of various evolutionary variables (*i.e.* evolutionary rates of the above-listed molecular traits) using available information on sequence, expression, gene annotation and protein localization from *Saccharomyces cerevisiae* and its homologous genes in related species ranging from *S. paradoxus* to *S. pombe*. We also compiled high-coverage functional genomic data on various genomic and functional properties of genes/proteins in *S. cerevisiae* (e.g. information on protein abundance, protein network connectivity, fitness contribution, genetic interaction connectivity, etc.). In addition to classical statistic tools, we employed a data mining regression tool (random forest) to predict evolutionary rates based on these gene properties. This enables us to compare the predictability and the main determinants of different aspects of molecular evolution.

First, we asked whether the different molecular traits of a gene evolve in a correlated fashion. We found that the different evolutionary rates show only very weak correlations with each other, suggesting that different gene properties diverge rather independently throughout evolution. Next, we examined the predictability of molecular evolution. Corroborating earlier reports, we found that sequence divergence is well-predictable, with 54% of variation in divergence rate explained. The rate of expression divergence and gene loss can also be predicted using genomic features (28% and 27%). Duplicability, however, showed little predictability. Furthermore, in contrast to other evolutionary traits, the rate of duplication is only marginally conserved when calculated on different branches of the phylogenetic tree. Taken together, these findings indicate that gene duplication is not driven by strong universal evolutionary forces. We found that the rate of evolutionary divergence in protein localization is also predictable and revealed novel factors determining the conservation of protein subcellular localization. For example, we found that highly expressed genes show especially strong conservation in localization.

In our study, we have systematically examined the driving forces behind evolutionary change of different gene-level molecular traits using data-mining methods and recent functional genomic datasets. While some evolutionary variables are highly predictable, we report that the diversity of duplicability across genes is lineage-specific and no strong universal determinant of duplicability exists. We also discovered a number of novel determinants of protein localization conservation.

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Analysis of blood cell lineages in *Drosophila melanogaster*

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Insects are armed with a powerful innate immune response, which provides an effective barrier against invaders and tumors. The phylogenetically conserved immune functions, such as the phagocytosis of microbes and the encapsulation of large foreign particles are carried

out by specialized immune cells, the hemocytes.

The development of the hemocyte lineages of *Drosophila melanogaster* is the result of a strictly regulated succession of intracellular and intercellular events. Studies on the *Drosophila* immune system have provided most of our knowledge on hematopoiesis and blood cell lineages in insects, and have shed light on some of the key features of blood cell differentiation in the animal kingdom.

The differentiation of hemocytes begins in the early embryonic stages. Two distinct mesodermal segments give rise to two independent embryonic hemocyte lineages: the procephalic mesoderm differentiates into embryonic macrophages and crystal cells, while the cardiogenic mesoderm forms the embryonic lymph gland. In the larval stages, hemocytes occupy three hematopoietic compartments. The lymph gland is a compact hematopoietic tissue consisting of paired lobes along the anterior end of the dorsal vessel. The sessile hematopoietic tissue localizes to the inner wall of the body cavity, and forms a banded pattern along the length of the larva. Both of these hematopoietic tissues contain differentiated effector cells, as well as precursor hemocytes. The third compartment, the circulation, comprises two effector hemocyte types: the plasmatocytes and the crystal cells. The plasmatocytes are phagocytic cells which engulf microbes and produce antimicrobial peptides, while crystal cells contain enzymes neccessary for the melanization cascade. Attack by the parasitoid wasp *Leptopilina boulardi* results in the appearance of a third effector cell class in the circulation, the lamellocytes, which form multilayered capsules around large foreign particles. Although the process of blood cell differentiation has been studied extensively, the origin of the hematopoietic compartments and effector hemocytes are still not well recognized.

Our aim was to track the distinct hemocyte lineages from the embryo to the adult and in the course of cell mediated immune response in *Drosophila*. In order to achieve this, we performed *in vivo* cell lineage tracing in combination with the use of molecular markers.

Our results show that the two embryonic hemocyte lineages form discrete larval hemocyte compartments, all contributing to the emergence of the effector cell pool upon immune induction. When we followed the fate of the effector cell types, it also became evident that plasmatocytes display a peculiar ability to transform into lamellocytes, therefore highlighting the plasticity of the effector hemocytes and the *Drosophila* immune system in general.

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Studying the function of the secretory pathway during germ cell formation especially of the Golgi associated retrograde protein (GARP) complex

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In many cases the malfunction of the sperm is the underlying factor in human fertility problems. However it is a significant issue, our knowledge is rather limited on the mechanism of the molecular structure and the development of the sperm.

It is well known that genes responsible for the phenotype and function of the sperm are evolutionarily conserved, including human, mouse and *Drosophila*. In the testis of *Drosophila* the whole process of spermatogenesis can be studied as from stem cell through every step to the mature sperm. During the last few years many membrane traffic mutants were identified with male sterile phenotype, what suggests that proteins involved in membrane trafficking has an important role to play in germ cell formation.

Vps54 (Scat) protein, a subunit of the Golgi associated retrograde protein (GARP) complex, is participating in the retrograde transport (transport from the extracellular space or organelles of the secretory and endosomal-lysosomal system towards the Golgi and the endoplasmatic reticulum).

Our research confirmed that Vps54 homozygous mutants show male sterile phenotype however females are fertile. Studying the mutant testis, abnormality could not be found at the early stages of spermatogenesis, however at later phases sperm cysts become unorganized and matured sperms were immobile.

Additionally we found that however viability of the homozygous Vps54 mutants were normal, the body-size of the mutants were conspicuously smaller according to the heterozygous ones.

Furthermore in flight tests mutants were found almost unable to fly and moreover, in larvae difficulties in peristaltic movement could be observed, what assumed defects in muscle or in neural system. Studying the muscle tissue of mutants although no structural aberration was found by fluorescent microscopy, loose myofibrils were visible with EM. For additional investigation of Vps54 mutant phenotype GFP, RFP and 6xMYC tagged Vps54 transgenic *Drosophila* lines were established to examine the localisation of the protein *in vivo* during spermatogenesis and myogenesis.

We do hope that our research contributes to gain a better insight into the function of Golgi associated retrograde protein transport during spermatogenesis. Also studies of GARP complex may open new perspectives as retrograde protein transport is scarcely investigated even so its importance is recognized.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Combined oxaloacetate and dehydroepiandrosterone treatment: a new neuroprotective startegy

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Stroke is accompanied by the development of neuronal and functional loss. Under ischemic brain pathological conditions interstitial glutamate (Glu) concentration increases to an excitotoxic level. Decreasing blood Glu concentration enhances the brain-to-blood efflux of Glu. The so called Glu scavenging from the brain moderates Glu excitotoxicity which contributes to the neuronal loss and long-lasting neurological deficits seen in stroke. Inflammatorical events take place in the affected area a few days after the excitotoxic period. In the aspect of therapeutic window Glu scavenging has to be done immediately after ischemic insult whereas antiinflammatory treatement is effective in the following 48 hours after cerebral ischemia.

4VO (four-vessel occlusion) as a global cerebral ischemic model was used to evaluate the neuroprotective effects of oxalocetate (OxAc) as a Glu scavenger and Dehydroepiandrosterone (DHEA) as antiinflammatic agent and the combined treatement.

ECoG recordings were carried out for the validation of the global ischemic intervention and for the detection of the effect of OxAc on post-ischemic ECoG pattern and on Burst-Supression ratio (BSR). Furthermore, power spectral density (PSD) and changes in ratio of frequency bands were measured. *In vitro* extracellular field-EPSP amplitudes were measured, LTP induction and I/O curves were recorded in the CA1 subfield of rat hippocampal brain slices.

FlouroJade C staining was used to visualize the degenerated CA1 neurons, Cresil-violet staining was used to estimate the thickness of the CA1 pyramid cell layer.

OxAc (20mg/100g bw) administered right after the ischemic insult decreased the formation rate of post-ischemic ECoG pattern. In the in vitro experiments both OxAc (20mg/100g bw) and DHEA (2mg/100g bw) resulted in a mild increase of the impaired synaptic plasticity in the CA1 region. The combined OxAc mediated glutamate scavenging and the DHEA treatement together were able to moderate the ischemic damage in the 4VO group and increased synaptic plasticity.

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The biosynthetic pathway of PGE2 and its role in the virulence of *Candida parapsilosis*

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Candida parapsilosis is often the second most commonly isolated *Candida* species from blood cultures, and it even outranks *Candida albicans* in some European, Asian, and South American hospitals. *C. parapsilosis* is an opportunistic human pathogen, that can colonize and cause disease on immuno-compromised patients (with AIDS, organ transplantation ect.) or in particular patient groups such as neonates or elders

Despite the increasing clinical importance, little is known about the virulence factors of *C. parapsilosis*. Thus, in our recent study we investigated the biosynthetic pathway of prostaglandin E2 (PGE2), a putative virulence factor of *C. parapsilosis*. Prostaglandins are fatty acid metabolites build up of 20 carbon atoms. Mammalians produce immune response regulator prostaglandins from arachidonic acid by the contribution of *COX1* and *COX2* cyclooxigenases. Although fungi do not possess cyclooxigenase homologes, several pathogenic species are able to produce prostaglandins from host originated arachidonic acids. In case of *C. albicans* the fatty acid desaturase homolog ole2 and the multicopper oxidase homolog fet3 enzymes were identified as potential key factors of the prostaglandin biosynthetic process. Due to its ability to block Th1-type, and promote Th2-type immune response, fungal Prostaglandin E2 can move the host's immune response towards helping the fungi to colonize and to carry out chronic inflammation. In our recent study we investigated the role of the putative fatty acid desaturase CpOle2 in the prostaglandin biosynthesis of the emerging human pathogen *C. parapsilosis*. We generated a homozygous *OLE2* deletion mutant through repeated application of a caSAT1 flipper KO cassette. We characterized the pseudohypha production, FBS utilization ability, growth ability on different pH and temperature of the *OLE2* deletion mutant in comparison to that of the wild type strain and we found that mutant strain showed the same characteristics as the wild type. First we characterized the prostaglandin profile of *C. albicans* and *C. parapsilosis* with HPLC and it showed that *C. parapsilosis* do produce PGE2, similarly to *C. albicans*, from the supplemented arachidonic acid. Than we purified *C. albicans* and *C. parapsilosis* PGE2 and we examined the immune modulating effect of these purified prostaglandins on human peripheral blood mononuclear cells derived macrophages (PBMC-DM) with the help of qRT-PCR. When the PGE2 production of *C. albicans* SC5314 and *C. parapsilosis* GA1 wild type, *CpOLE2* heterozygous deletion *(ole2 /OLE2*) and homozygous deletion mutant (*ole2 /ole2*) was measured after the treatment of arachidonic acid by Enzyme-linked immunosorbent assay

(ELISA) we found no difference in the PGE2 production of the mutant strains compared to the wild type strain. Although the C. albicans *OLE2* gene proved to be participating in the PGE2 production, these results are intending that the *CpOLE2* gene do not play a role in the *C. parapsilosis* PGE2 biosynthesis. In order to identify genes that play role in the *C. parapsilosis* PGE2 biosynthesis we carried out qRT-PCR on several genes, chosen from literature and by *in silico* work, after treatment of arachidonic acid. The analysis revealed the significant up regulation of the potential multicopper ferro-O2-oxidoreductase (*CpFET3*) gene after the induction of arachidonic acid. Henceforth while we are creating the *CpFET3* homozygous deletion mutant, we are intending to identify further genes that participate in the PGE2 biosynthetic pathway by carrying out a micro-array analysis.

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Role of the HLTF in the tumorigenesis

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The helicase-like transcription factor (HLTF) belongs to the SWI/SNF family of chromatin-remodeling factors. Several SWI/SNF genes are disrupted in cancer, suggesting their possible role as tumor suppressors. Similarly, the HLTF gene was found to be inactivated by hypermethylation in a significant number of colon, gastric and uterine tumors, indicating that HLTF silencing may confer a growth advantage and that HLTF could be considered as a tumor suppressor gene.

Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of K-RAS and BRAF is central to the progression of colorectal cancer. According to published data the K-RAS mutation may be present in 35%-45% of patients with colorectal cancer.

The HLTF gene was silenced by hypermethylation in 45% of colon cancers. Recent studies did not examine the connection between HLTF and other tumor suppressor genes and oncogenes.

The K-RAS mutation and HLTF promoter hypermethylation can be detected equal frequency in colon cancers, but the correlation between them is not examined yet.

We examined about 100 colorectal cancer samples. We analyzed the K-RAS status, the microsatellite status and HLTF promoter hypermethylation. Our results indicate that there are some correlation between the K-RAS mutation and the HLTF promoter hypermethylation. Additionally there is correlation between HLTF hypermethylation and microsatellite instability.

The DNA Mismatch Repair (MMR) system plays an essential role in maintaining the fidelity of DNA replication by correcting single nucleotide mismatches and insertion/deletion (ID)-loops. Many components of eukaryotic MMR have been identified, the molecular mechanism of MMR has been largely demonstrated. Defects in the mismatch repair system result in a mutator phenotype, manifesting as microsatellite instability (MSI) in DNA of affected cells

Recently it has been published that many DNA repair genes (e.g. RecQ helicases, polymarese κ and HLTF) are possible new players of the MMR. We are developing a new useful research tool to verify the contribution of these potential candidates in the MMR process.

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The Role of *Candida parapsilosis* Secreted Aspartyl Proteinase 1 in Host-Pathogen Interactions

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Yeast of the genus *Candida* remain the most prevalent cause of human mycotic diseases worldwide and range in severity from superficial infections to life-threatening systemic diseases. *Candida parapsilosis* is currently the second most common cause of invasive candidiasis. Adhesion to host surface, cell morphology switching between yeast and filamentous growth, biofilm formation and secretion of hydrolytic enzymes such as lipases, phospholipases and aspartyl proteinases are considered to be the most important features for development of candidial disease. *C. albicans* possesses 10 genes encoding secreted aspartyl proteinases (Saps) where expression of a particular Sap isoenzyme depends on the type, site and stage of infection. In contrast little is known about the exact role of *C. parapsilosis* secreted aspartyl proteinases (Sapps) in the development of virulence in host-pathogen interactions. In *C. parapsilosis SAPP1* and *SAPP2* are the 2 annotated secreted aspartyl proteinase genes. Sapp1 and Sapp2 have been studied at the enzymological level. The production of Sapp1 in inducer medium is

at least one order of magnitude higher compared to Sapp2. As with *C. albicans* Saps, both *C. parapsilosis* Sapp proteins are synthesized as preproenzymes and can be activated autocatalytically or by a membrane-bound Kex2-like protein. It has been previously demonstrated that the epidermal and epithelial damage caused by *C. parapsilosis* in reconstituted human tissue was significantly reduced in the presence of the proteinase inhibitor pepstatin A, that suggested that *C. parapsilosis* Sapps are involved in virulence.

In this study, we analyzed the role of Sapp1 in virulence. The *in silico* analysis of *SAPP1* sequence revealed a 2871bp duplicated region (*SAPP1a* and *SAPP1b*) in the genome of *C. parapsilosis*. With the help of the *caSAT1* flipper cassette system we generated homozygous ΔΔ*sapp1a*, ΔΔ*sapp1b* and ΔΔ*sapp1a*-ΔΔ*sapp1b* mutants. Sapp1 production in an inducer medium was reduced by approximately 50% in the ΔΔ*sapp1a* and ΔΔ*sapp1b* mutants but the production of Sapp2 was nat affected. In contrast, Sapp2 production was increased in the ΔΔ*sapp1a*-ΔΔ*sapp1b* mutant relative to the wild type (WT) strain. The ΔΔ*sapp1a*-ΔΔ*sapp1b* strain was hypersusceptible to human serum and was attenuated in its capacity to damage host-effector cells. The phagocytosis and killing of ΔΔ*sapp1a*-ΔΔ*sapp1b* yeasts by human peripherial blood mononuclear cells (PBMCs) and PBMC-derived macrophages (PBMC-DM) was significantly enhanched relative to WT. Phagolysosomal fusion in PBMC-DMs occured more than twice as frequently with ingested ΔΔ*sapp1a*-ΔΔ*sapp1b* yeast cells compared to WT.

All these data suggests that *C. parapsilosis* Sapp1 is an important virulence factor, since it is associated with the capacity of the fungus to grow in human serum and to survive inside macrophages, and this particular proteinase can be a potential target for the development of new antifungal drugs.

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Oxidative stress/antioxidant defense system

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Oxidative stress involves a shift towards the pro-oxidant in the pro-oxidant/antioxidant balance, which can occur as a result of an increase in oxidative metabolism. It is well known that reactive free radicals lead to a number of damaging effects as they can attack lipids, proteins, carbohydrates and DNA in cells as a consequence of various factors, including exposure to heavy metals, medication, toxins or surgical interventions. To protect against reactive oxygen species and other toxic materials that can generate oxidative stress, aerobic organisms have evolved a complex antioxidant defense systems. During the stress response, molecular processes are activated, which help to restore / remove the damaged molecules, to make the cells temporarily more resistant against the stressor. Some components of the response, including activation of the endogenous antioxidant defense system, are highly preserved throughout the evolution. The major aim of the work in our research group is to study the molecular mechanisms leading to the activation of the antioxidant defense system.

We have identified and studied numerous members of this coordinated system, such as antioxidant enzymes, metal-binding proteins and low molecular weight antioxidants. In two model organisms, three different approaches were used to induce an increased level of free radical formation: we studied heavy metal-induced changes in fish, and followed the changes caused by Streptozotocin induced diabetes and endotoxin induced inflammation in rat models.

In this study, we have focused on two quite different (in structure and operation mechanism) protein families: heme-oxygenases (HOs) and metallothioneins (MTs) with several isoforms. HOs are rate-limiting enzymes in the heme catabolic pathway. HOs play roles in heme degradation, and also produce carbon monoxide, a vasoactive dilator agent with important free radical scavenging properties. Two major isoforms of HO have been characterized: HO-1, which is inducible in response to stressors, such as heavy metals, oxidative stress and cytokines, and the constitutively expressed HO-2. The MTs are a family of low–molecular weight metal-binding proteins. These non-enzymatic intracellular proteins are characterized by their unusual high cysteine contents. The MTs are involved in the detoxification of certain heavy metals, the homeostasis of essential trace elements and the scavenging of free radicals. Consistently with these roles, MT genes in eukaryotes are transcriptionally induced by a variety of stressors, including metals, hormones, oxidative agents, cold exposure and irradiation. Though the regulations of the HO and MT genes differ substantially and their expression is regulated temporarily and spatially (which may suggest distinct physiological roles), there are common inductors of these genes, including metal- and antioxidant-responsive elements in their promoter regions.

To summarize our results, it may be concluded that the HO and MT genes are induced in all the studied systems. Elevated levels of expression of HO and MT isoforms are observed in all models; their expression demonstrates stressor-, isoform- and tissue-specificity. As mentioned above, the members of the two gene family have very different characters and regulations, however in the three tested models, the control of MT and HO genes are finely coordinated and they are induced in a complementary manner.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

A new regulating protein of the ubiquitylation of human PCNA

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Stalled replication machinery on the DNA is a critical threat to the cell, since it can collapse, leading to the accumulation of genetic changes or cell death. Stalling can occur when the replicative polymerase is unable to process beyond a particular point for any reason, such as when DNA damage is encountered through which the polymerase cannot replicate. Upon stalling of the replication fork cell will die if there is no resolution to this problem. However, there are several strategies that the cell may employ to rescue the replication fork. These are often collectively called damage tolerance pathways, since the lesion is not repaired, but "tolerated" as the cell finds a way to overcome the defect of replication stalling. These mechanisms include DNA damage bypass, homologous recombination (HR)-dependent repair and non-homologous end-joining (NHEJ)-dependent repair to deal with fork collapse. Although replication stalls frequently a delicate balance of damage bypass, homologous recombination and non-homologous end-joining could ensure survival and at the same time effectively prevent increased mutagenesis, gross chromosomal rearrangement, and carcinogenesis.

Genomic instability has been documented as a preceding step for multiple inactivations of tumor suppressor genes and activations of proto-oncogenes that can lead to cancer. In our study we are focusing on the regulation of the ubiquitylation of PCNA to give more insight into the regulation of DNA damage tolerance pathways. We identified a new player which has role in regulation of PCNA ubiquitylation.

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Changes in birth weight and developmental status of newborns in 20 years in Szeged

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The developmental status of a newborn baby depends on several factors, but mostly on the physical status of the mother and her lifestyle, and living circumstances.

Therefore the monitoring of the changes of birth data is necessary to understand better the changes of the health status of a nation. Both high and low birth weight means risk factor for the neonate and the mother also. Several studies pointed out that there is an acceleration of birthweight parallel with the growing rate of obesity, and the two can have connections. Studying the changes of the developmental status of newborns offers us a special view of the health status of Hungary. Socio-economical changes of Hungary since the change of the system in 1989 have an indirect impact on the health status of Hungary – which can be measured by health and developmental status of newborns.

Data of newbornes born in 1989, 1999, 2004 and 2009 were collected at the Obstetrics and Gynaecology Department, University of Szeged. Body sizes were measured by clinical staff. Data of mothers and pregnancy were collected while admission to the hospital. We studied the singleton birth in three categories according to birth weight. Birth weight categories were set the followings: 0-2499g: small birth weight, 2500-3999g: normal birth weight, and 4000-6000g: macrosom babies. Statistical analysis was carried out with Microsoft Excel and SPSS 14.0. We assigned the level of significance at 0.05.

In the four studied year 8430 babies were born. Among 8142 singleton birth 6784 babies (83.31%) were born with normal birth weight, and 626 babies (7.68%) were macrosoms. In 20 years the mean average of maternal age, birth weight of babies are rising, and are significantly different in the four years when counting all newborns. The maternal age rose with 3.58 yr, the average bodyweight rose with 77.72g in the normal bodyweight group, the difference is significant among the 4 years averages. Numbers of birth, and cesarean section rose too. Birth weight rose significantly both by boys and girls of 20 yrs perspective. In both gender there is a significant change of bodyweight, bodylength, and maternal age. In the macrosom group we found significant difference in the mean of maternal age in both gender, but in bodylength just in the boy group, and in length of gravidity in the girl group.

We found a rising rate in birth weight and maternal age in our sample collected at the Department of Obstetrics and Gynaecology, University of Szeged. The changing rate is typical in almost all western countries, and suggests it is worth to pay attention to in Hungary as well. Change of lifestyle since the change of the system is visible in our sample also by the growing rate in almost all of our factors. The growing frequency of overweight and obesity in Hungary too may have showed its impact on birth weight also.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Significance of orexin in the water metabolism and the regulation of vasopressin secretion

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Orexins have been described before a decade, from the lateral hypothalamic area. Orexin neurons project to multiple region in the brain, including the hypothalamic paraventricular and supraoptic nucleus, which are the main vasopressin (VP) producing cells in the central nervous system. Positive immunostaning of the orexin receptors (OX_1R and OX_2R) were also observed in these magnocellular regions. The influence of the orexins on the water metabolism has been proved, but the possible role of VP release in connection with polydipsia and polyuria has not been clarified.

The effects of the centrally administered neuropeptides orexin-A and -B on water intake and VP secretion after hyperosmotic and histamine (HA)-induced stimulus were studied *in vivo* in male Wistar rats*;* and the effects of monoamine (dopamine (DA), serotonin (5-HT), HA, adrenaline (ADR), noradrenaline (NADR)) and K^+ administration on VP secretion were studied *in vitro* in 13-14-day cell cultures from rat neurohypophysis (NH), and it was examined whether orexins can modify the induced VP release enhancement.

Increased water consumption was observed after the administration of both orexin-A or orexin-B. There were no changes in basal VP concentration of the plasma after the administration of different doses of the orexins. A significant increase in VP secretion was detected following HA and 2.5% NaCl administration, a moderate VP level enhancement was detected in the latter case. Centrally administered orexin-A blocked the VP level increases induced by HA or hyperosmosis. The inhibitory effects of orexin-A were prevented by specific OX_1R antagonist.

Following administration of orexin-A or orexin-B in increasing doses, significant changes were not observed in the VP levels of the supernatant media of the cell cultures from isolated rat NH. VP level substantially increased after NADR, ADR or 5-HT treatment, while the enhancing effects of DA, HA or K^+ administration were more moderate. Preincubation with orexin-A or orexin-B reduced the monamine-induced VP level increases, except in the case of DA. The decreases were significant, but the VP concentrations of the supernatant media remained high above the control level. There was no significant difference in the decreasing effect between orexin-A and orexin-B. Orexins had no influence on the VP level increase induced by K^+, which causes non-specific, receptor-independent hormone secretion. Orexin-A or -B did not induce any changes in VP release when administered after the monoamine-treatments. OX_1R antagonist treatment avoided the effects of the orexin-A preincubation on monoamine-induced VP level enhancements.

According to our results we concluded that: 1. Orexin-A or orexin-B can cause polydipsia. 2. Orexin-A (when administered i.c.v. *in vivo*) and both orexin-A or orexin-B (by preincubation in cell cultures) can reduce the induced VP release enhancement. 3. The effects of orexin-A on the water metabolism or on the VP level increases (either histamine- or osmotic-induced *in vivo,* or monoamine-induced in NH cell cultures) are mediated through the OX_1R. 4. The interactions of the orexin systems regarding VP secretion occur both at hypothalamic and at the level of the posterior pituitary.

Supported by SROP 4.2.2.-08/1-2008-0006, TÁMOP 4.2.1/B-09/1/KONV-2010-0005, HURO/0901/037/2.2.2.

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Genetic analysis of the FMRFamide-related neuropeptides and their specific receptors in *Drosophila melanogaster*

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Neuropeptides are produced and secreted by specific neurons in all Metazoan organisms. They steer important processes like reproduction, feeding, behaviour, circadian rhythm, etc. from worms to humans. They are also very important regulators of insect life. *Drosophila melanogaster* has 45 known neuropeptides. We use the excellent genetic system of the fruitfly to perform a systematic genetic analysis of the functions exerted by the FMRFa-related (FaRP) group of peptides (FMRFa, Dms, Dsk, NPF, sNPF) and their GPCR receptors (FR, Dms-R1 and -2, Dsk-R1 and -2, NPFR, sNPFR).

We have built a RNAi-based genetic system in which the FMRFa-related genes and their specific receptors can be silenced in pairwise combinations. For this we used the RNAi transgenes available from stock centers. The RNAi transgenes can be driven by the Gal4-inducible UAS promoter. Using the Act5-Gal4 „driver" which induced an ubiquitous and continuous expression of the RNAi double-stranded RNA, silencing of the *FMRFa, Dms, Dms-R1* and *Dsk-R2* genes resulted in complete lethality while the others remained viable. The lethal effect

was observed with the KK series of mutants where the silencing construct is inserted into a standard „landing site" in the 2nd chromosome ensuring an uniform strong expression. The same constructs inserted at accidental places (GD series) had no lethal effect, probably due to lower expression.

By P transposon remobilization, we isolated 8 intragenic deletion mutants in the *DMS-R1* gene which abolished the gene function. However, in contradiction to the RNAi results above, the homozygous mutant flies proved to be fully viable and fertile. The contradiction could be explained with the „off-target" effect: if the RNAi construct contains a ≥20-mer sequence motif shared by a non-target gene, both genes will be silenced, and the lethality can come from the non-specific off-target effect. Another possibility is that the lethality is caused by the Act-Gal4 driver activating the gene expression at tissues or organs in which the gene is not normally expressed. These possibilities are investigated now by using more specific drivers, e.g. elav-Gal4 which is restricted to the CNS neurons.

It is known that the 5' upstream DNA sequence regulates the expression pattern of the *FMRFa* gene in the *Drosophila* CNS (Benveniste and Taghert, J. Neurobiol. 38, 507-520), and certain parts of this sequence can reproduce specific parts of the pattern.We amplified by PCR these DNA sequences, cloned them into the pBPGUw vector upstream to the Gal4 coding sequence, and made transgenic stocks carrying these constructs. These new drivers can be used in future experiments to target UAS-dependent expression to the FMRFa-expressing neurons.

In cooperation with Dr.Michal Žurovec (Inst. of Entomology, Czech Acad. Sci., České-Budejovice) we test the above mutant combinations for their basic metabolism (CO_2 production) and moving activity. In CO_2 production there was no difference between the mutant and the wild type adults. However, the mutants showed significantly less moving activity than the wild type. Experiments to test the possible mutant effect on heartbeat frequency are also in progress.

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Biogas production from protein rich substrates

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In the European Union currently there are approximately 6000-7000 biogas plants, none of them processes protein-rich waste. However, in the course of food production and processing many protein-rich by-products and waste are made. These are serious pollutants, their disposal is a costly and energy-consuming task. In the world millions of tons of protein-rich hazardous waste are produced continuously. Biogas is a renewable energy carrier and the production of biogas is associated with double benefits: elimination of environmental pollution problems is coupled with the generation of renewable energy carrier. The digestion effluent is used as fertilizer for agricultural nutrient recovery and eliminates the need for artificial fertilizers, the production of which is a highly energy demanding process. Anaerobic digestion of slaughterhouse waste presents a specific task because this waste stream is rich in proteins. Blood, casein and meat powder have a very low C/N ratio therefore they are not favorable substrates for biogas production. Several earlier attempts corroborated the inhibitory effects of elevated NH_3 – NH_4^+ concentrations on anaerobic digestion.

Anaerobic digestion of animal waste was investigated in batch and continuously stirred tank reactor experiments at 37°C. In all experiments efficient degradation of blood, casein and meat-powder containing samples were observed using a specially adapted microbiological consortium. Contrary to the findings published earlier ammonia did not inhibit the biogas process at concentrations up to 10 g N/dm^3.

As pH raises the free ammonia concentration increases significantly. The experiments were designed to compare the protein hydrolysis potential of substrates that were acclimated and non-acclimated to protein rich media.

Proteinase activities of the consortia were monitored regularly. The changes in acetate and ammonium-nitrogen concentrations were followed during the fermentations. Volatile fatty acid compositions (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic acids) were determined using HPLC to monitor the microbiological activity in the reactors. Total carbon and total organic carbon contents have been measured to determine the C/N ratio of the biomass. Volumetric biogas yields gave information about the efficacy of the anaerobic digestion process. The composition of the evolved gas was determined by gas chromatography.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Mycolic acid biosynthesis in *Rhodococcus erythropolis*

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Oil contamination is one of the most hazardous environmental pollutions. There were numerous oil spills in the last three decades. Oil tanker accidents and oil-rig catastrophes are still reported. Oil spills had great impact on the wildlife as well as on the economy by cutting down the agriculture, food industry and tourism.

Surfactants are useful weapons in the war against oil pollution. They are suitable to solubilize hydrophobic materials, consequently to clean oil tanks and pipes. In addition they can be used for emulsification of animal fats in food industrial and housekeeping wastewaters. Many bacteria can produce substantial amount of biosurfactants which can promote the solubilization of hydrophobic hydrocarbons, thus these bacteria themselves or the native microflora can utilize the unctuous pollutants. An additional advantage of the biosurfactants over the synthetic surface active molecules is that these compounds are easily biodegradable, they don't persist in the environment.

A special biosurfactant group is composed of mycolic acids which are basically α-alkyl, β-hydroxy fatty acids. Mycolic acids are the most characteristic components of the cell wall of the so called mycolata bacterial group. This group belongs to the Actinomycetales and contains the genera *Mycobacterium*, *Corynebacterium*, *Nocardia*, *Rhodococcus* and others.

A Gram+ bacterium, Rhodococcus erythropolis MK1 strain has been isolated from polluted soil in our lab. The cells grown in the precence of hydrocarbons produced cell-wall-bound surfactants in order to make oils accessible. Based on the literature and preliminary gas chromatographic analysis, this surface active compound seemed to be trehalomycolate. Although, the mycolic acid biosynthesis was already characterized in the pathogenic Mycobacteria, the biosynthetic routes of trehalomycolate in Rhodococcales are less known. Therefore, we aimed to map the mycolic acid biosynthesis pathway in Rhodococcus erythropolis strains. First, we sequenced the genome of our strain by SOLID™ next generation DNA sequencer. The reads were mapped on the R. erythropolis PR4 genome in the NCBI database. We searched for rhodococcal homologs of the known mycobacterial and corynebacterial genes involved in mycolic acid biosynthesis. We found conserved regions in the genome which are likely responsible for the biosynthesis of mycolic acids. Nevertheless, differences in the biosynthetic pathways can also be recognized. The ongoing comparative whole genome transcript analysis discloses the genes really necessary for the anabolism of trehalomycolates. This knowledge will be used for constructing strains for biotechnological applications.

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Chronic hypertriglyceridemia induces early tau hyperphosphorylation and impaired long term potentiation in apoB-100 transgenic mice

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Alzheimer's disease (AD) is the commonest form of dementia and affects more than 32 million people worldwide. Epidemiological studies confirmed the importance of vascular dementia as the second most common cause of dementia in the elderly, representing 15–20% of all cases of dementia. These figures threaten to rise as the population ages unless efficient preventative measures are introduced.

The role of hyperlipidemia in the development of vascular dementia and cognitive decline is still controversial. Recent studies indicate that ApoB-100-induced hyperlipidemia and atherosclerosis are not only implicated in the pathogenesis of cardiovascular diseases but may also affect the cerebrovascular system thus contribute to the development of neurodegenerative disorders. Other studies have shown that AD is accompanied by an elevation in apolipoprotein B concentration in the serum. It was also demonstrated, that progressive cognitive impairment in AD is accompanied by neurovascular dysfunction.

To further clarify the possible role of hyperlipidemia in the development of neurodegeneration and dementia we have generated transgenic mice overexpressing the human ApoB-100 protein. Transgenic mice fed a regular chow diet show elevated serum triglyceride levels, fed a cholesterol rich diet show hypercholesterolemia leading to coronary and systemic atherosclerosis by the age of 6 months. Previously, we have shown that microcapillary density significantly decreases in the cortex of hyperlipidemic (hypertriglyceridemic and hypercholesterolemic) transgenic mice.

Here we show, that adult transgenic mice (6 months old) present significantly elevated cerebral level of triglycerides and ApoB-100 indicating the dysfunction of blood-brain barrier. Moreover, in aging transgenic mice (10 months old) triglyceride-rich lipid-droplets in the cortex can be detected. The level of ApoE, the major lipid transporter molecule in the brain was also significantly increased in adult transgenic brains. Under pathological conditions (e.g. AD) the Tau protein becomes hyperphosphorylated, which leads to dynamic instability and disintegration of microtubular network and eventually to the formation of neurofibrillary tangles (NFT). Tau phosphorylation patterns in the brain of 3 and 6 month old transgenic animals were investigated using several phosphosite-specific antibodies (Ser^{199}, $Ser199/^{202}$, Ser^{262},

Ser^{396} and Ser^{404}) which are commonly used in the molecular diagnosis of AD. We demonstrate here, that Tau protein is hyperphosphorylated at sites Ser^{199}, Ser^{202}, Ser^{262}, Ser^{396} and Ser^{404} in the cortex of 6 month old hypertriglyceridemic transgenic mice. Neuronal apoptosis was monitored in transgenic brains using FluoroJade staining. Significantly increased degenerated neurons were counted in the cortical and hippocampal regions of adult transgenic versus wild-type mice. Furthermore, using electrophysiological recordings (long-term potentiation and paired pulse facilitation) we demonstrated severe impairment in presynaptic function of transgenic brains.

These results indicate that chronic hypertriglyceridemia can induce neurodegeneration possibly via hyperphosphorylation of tau protein. Beyond of theoretical observations we aim to develop a novel mouse model of age-related neurodegeneration, providing a useful tool for the development of efficient therapies against neurodegenerative diseases.

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Examination of real-time effects of different cytotoxic and cytoprotective compounds with a novel cell-microelectronic sensing technique

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Real-Time Cell Analyzer (RTCA) DP is a novel cell migration and invasion assay system that uses the Boyden Chamber principle but does not involve any fixation, labeling or counting of the cells. The core of the system is the CIM-Plate device, composed of an upper chamber and a lower chamber. The upper chamber has 16 wells that are sealed at the bottom with a micro-pore-containing polycarbonate or polyester membrane. The membrane contains microelectronic sensor arrays that are integrated on its bottom surface. Migration of cells will occur through these electrodes, which changes impedance, and will increase cell index. The more cells migrate the higher the cell index will be. RTCA SP is also a microelectronic cell sensor method, where microlectrodes are integrated in the bottom of a microtiter plate (96-well E-plate) and measures adhesion, proliferation or cytoxicity. The real-time measurement can detect changes continuously, which means that the system can give information at any stages of the experiment.

We examined the effects of cytotoxic compounds on the migration and proliferation properties of human glioblastoma, liver carcinoma and melanoma cells with a novel cell microelectronic sensing technique. We tested the migration potential of several tumor cell lines in a trans-well migration system. In addition we examined the effects of neuro/cytoprotective compounds on the viability of primary rat neuronal cultures with RTCA-SP system. During the cytotoxicity and cytoprotection screenings *in vitro* cytotoxicity or cytoprotectivity was elicited by numerous compounds synthesized by Avidin Ltd.

In our experiment we examined the migration properties of different tumor cells. GBM3 human glioblastoma cells migrated the fastest. A549 human adenocarcinomic lung cancer cells also showed a relatively high cell index increase, probably due to its small size, enabling it to pass easily through the membrane. Ac929 treatment decreased migration ability of Hep3B hepatoma cells dose-dependently. The highest concentration used (1 μM) resulted the lowest cell index. U87-MG human glioblastoma-astrocytoma cells were treated with Ac1041and Ac915 compounds. The treatment caused dose-dependent decrease of cell index, where 500 nM and 1 μM concentrations were ineffective. 5 μM showed a slight change in migration, and higher doses (20-50 μM) were cytotoxic. Cell index data were calculated 24 hours after treatment. The experiments clearly shows the dose-dependent effect of Ac-compounds. Effects of Ac1041, Ac929 and Ac915 were validated by the RTCA SP system.

During the cytoprotective screenings in vitro cytotoxicity was elicited by hydrogen peroxide in primary rat neuronal cultures. Cells were either pre-treated 5 min before oxidative stress or post-treated at 30 min with novel cytoprotective compounds**.** Cell Index of Q2 treated cells started to rise as high as absolute control and remained elevated for hours, showing a long-term cytoprotective effect. Vehicle-control cells (which received H_2O_2 and vehicle, but no treatment with cytoprotective compounds), showed a rapid decline of cell index. Pre-treatment with compound 9791 or post-treatment of the cells with the fatty acid derivative 9528 prevented the cells from the toxic effects of oxidative stress dose-dependently.

The cell-microelectronic sensing technique (RT-CES) method is suitable for the screening of molecular libraries to identify molecules or molecule combinations that attenuate oxidative stress-induced cell damage and can also be useful for screening of agents with antitumor properties.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Ascorbate, as alternative electron donor to photosystem II, protects plants against photoinhibition and stimulates the photoproduction of hydrogen in green algae

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Oxygenic photosynthetic organisms produce organic materials by using light energy and water as terminal electron donor. The water splitting enzyme, *i.e.* oxygen evolving complex (OEC) is one of the most vulnerable components of the photosynthetic electron transport chain. It has been shown (Tóth et al. 2007, Biochim Biophys Acta 1767: 295-305) that if the OEC is damaged, alternative electron donors, present in large amounts, donate electrons to photosystem II (PSII) in vivo. Our studies carried out on higher plant leaves and algal cells have shown that this alternative electron donor molecule is ascorbate; the rate of electron donation depends on the ascorbate content of leaves: $t_{1/2}$ is approximately 25 ms in wild type Arabidopsis plants and about 55 ms in ascorbate-deficient mutants (Tóth et al. 2009 Plant Physiol. 149: 1568-1578).

When OEC is damaged by heat stress highly oxidising components (Tyr_Z^+ and $P680^+$) accumulate in PSII in the light, leading to the fast inactivation of PSII. We have demonstrated that under these conditions ascorbate has a protective function by providing electrons to PSII and slowing down the harmful accumulation of Tyr_Z^+ and $P680^+$ (Tóth et al. 2011 Plant Physiol. 156:382–392).

Based on these results, it was reasonable to assume that ascorbate, by replacing the water splitting enzyme and supporting the electron transport without oxygen evolution, can enhance the photoproduction of hydrogen in *Chlamydomonas reinhardtii* cells. Photoproduction of hydrogen is known to depend on the activity of PSII; however, the oxygen evolution associated with PSII activity strongly inhibits the hydrogenase. It has earlier been shown that Chlamydomonas cells are able to evolve considerable amounts of hydrogen under anaerobic conditions following their sulphur deprivation, which suppresses their PSII activity (Melis and Happe 2001; 127:740–748). Our experiments have shown that the addition of 10 mM ascorbate to sulphur-deprived cell culture accelerates significantly the linear electron transport via PSII to PSI and to the hydrogenase, leading to a three-fold increase in hydrogen production. Similar results were obtained by using diphenylcarbazide (DPC), an artificial electron donor to PSII. The stimulation of hydrogen production was sensitive to diuron and dibromothymoquinone (inhibitors of PSII and the cytochrome b_6f complex, respectively), which proves that the enhancement of the hydrogen evolution by ascorbate and DPC can indeed be accounted for by their functioning as alternative PSII electron donors.

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Characterisation of host-pathogen interaction during *Candida* infections

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Candida species are known as members of the normal human flora. However under certain circumstances these commensalist yeasts are able to transform themselves into opportunistic pathogens. *C. parapsilosis* is considered to be the second or third most common *Candida* species causing candidiasis after *C. albicans*. The response of the mammalian immune system given to the *C. albicans* is well-exemined, and based on our pervious work it is clear, that some *Candida* derived lipases play role as virulence factor. On the other hand little is known on the interaction between the immune system and other *Candida* species, like *C. parapsilosis*.

We previously showed that *C. parapsilosis* lipase knockout (LIP-) mutants were significantly deficient in their capacity to produce biofilm, to grow in lipid rich medium, and to survive in macrophages. In an attempt to understand this reduced virulence phenotype, we developed an *in vitro* model system using murine macrophage -like cell line J774.2. We examined the gene expression in J774.2 macrophages infected with wild type (WT) *C. parapsilosis* and LIP- cells. The complex response of murine macrophages to infection with *C. parapsilosis* was investigated at the level of gene expression using Agilent mouse microarray. 155 and 512 genes were identified as being differentially regulated at 3 and 8 hours post infection, respectively. Most of the upregulated genes encoded molecules that were involved in immune response and inflammation, transcription, signalling, apoptosis, cell cycle, electron transport and cell adhesion. Of particular interest were the upregulation of proinflammatory cytokines, typical of the classically activated macrophages such as TNF, IL-1 and IL-15, and also the upregulation of TNF-receptor family members such as *TNFRSF9* associated with Th1 T-helper cell responses. Additionally, the microarray data indicate significant differences between the response to *C. parapsilosis* infection and that of *C. albicans*.

Flow cytometry analysis proved, that elevated mRNA level of TNFRSF9 correlated to the elevated amount of protein on the surface of J774.2 macrophage cells upon *C. parapsilosis* WT infection. Similar pattern of TNFRSF9 (CD137) regulation could be observed in cells from whole human blood upon *C. parapsilosis* WT infection. To further examine the host pathogen interactions we established a human monocyte (THP-1) cell line infection model. THP-1 cells were infected with eight different *Candida* strains from the parapsilosis *sensu lato*

group and subjected for complete transcriptome analysis. In order to profile the transcriptome changes with the best possible resolution, we utilized the robustness and accuracy of the Next-generation Sequencing (NGS) RNA-seq technology.

To further develop our infection models we established an in vitro system using primary human mononuclear blood cells. Monocyte-derived immature and mature dendritic cells (iDCs, mDCs) as well as macrophages (MΦ) co-cultured with live WT or LIP- *C. parapsilosis* strains were studied to determine the host response. We determined that all cell types efficiently phagocytosed and killed *C. parapsilosis*, furthermore our results show that the phagocytic and fungicidal activities of both iDCs and mDCs are more potent for LIP- compared to WT yeast cells. Notably, MΦ showed eleveted fungal killing activity to LIP- cells but no increased phagocitic capacity was detectable. In addition, the LIP- *C. parapsilosis* cells induce higher gene expression and protein secretion of proinflammatory cytokines and chemokines in all cell types relative to the effect of co-culture with WT yeast cells. Our results show that both DCs and MΦ are activated by exposure to *C. parapsilosis*, as shown by increased phagocytosis, killing and proinflammatory protein secretion. Moreover, these data strongly suggest that *C. parapsilosis* derived lipase has a protective role during yeast:phagocyte interactions, since lipase production in wt yeast cells decreased the phagocytic capacity (in case of DCs) and killing efficiency of host cells and downregulated the expression of host effector molecules.

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The role of guard cell photosynthesis in biotic stress-induced stomatal closure

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Guard cells (GCs) control gas exchange and transpirational water loss of leaves by turgor-driven volume changes. Environmental and hormonal signals regulate opening and closure by activating diverse signalisation networks and membrane transporters. GCs also respond to the presence of microbes following perception of microbe-associated molecular patterns, such as a fungal elicitor chitosan (CHT). It has been shown that CHT inhibits the blue light-induced stomatal opening and can trigger stomatal closure through distinct signaling pathways and transporters. Stomatal opening and closure is related to the H^+-ATPase activity in the GC plasma membrane, as it affects the transport of osmotically active solutes. ATP for proton pumping is partly supplied from photophosphorylation in *Vicia faba* GCs (Mawson 1993). In order to investigate whether CHT affects the photosynthetic ATP production, the light-dependence of the photosynthetic electron transport rate of individual GCs was assayed. We found that when CHT was applied before sunrise, the apparent relative linear electron transport rate (ETR) remained low contrary to control. However, when CHT was sprayed on leaves by day, it only induced slight stomatal closure without a significant change of these photosynthetic parameters. CHT was shown to induce the generation of both reactive oxygen and nitrogen species in pea GCs (Srivastava et al. 2009). Using fluorescent probes we found that one hour of CHT treatment led to a significant increase in both hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels of *Vicia* GC chloroplasts. H_2O_2 accumulated mainly in chloroplast stroma and nucleus, while NO was found in the cytosol and chloroplasts. We also found that the inhibitory effect of CHT on the morning photosynthesis can be mimicked by exogenously applied NO, therefore it can be hypothesized that CHT acts through the NO signaling pathway.

NO triggers the release of Ca^{2+} from intracellular stores in GCs, which leads to stomatal closure. NO may also have an indirect effect through the decrease of intracellular ATP level generated by GC chloroplasts. Earlier we showed that NO slows down electron transfer between Q_A and Q_B, and inhibits charge recombination reactions of Q_A^- with the S_2 state of the water-oxidizing complex in pea leaves (Wodala et al. 2008). The microscopy version of a pulse-amplitude-modulated chlorophyll fluorometer (PAM) combined with a rapid solution exchanger allowed us to monitor the photosynthetic activity of a GC before and after the addition and also rapid removal of NO. The concentration of NO released from GSNO under light was measured in a solution entering the recording chamber using a NO-electrode. We found that NO decreases the electron transport rate resulting in a modest acidification of the thylakoid lumen and conceivably a reduced ATP synthesis. Variable fluorescence yield (Fv) was increased immediately in a biphasic manner after the inflow of the NO-containing solution, in line with the kinetic differences in the changes of photochemical and non-photochemical quenching. The wash-out of NO resulted in a sudden decrease of Fv, which was further accelerated by bicarbonate, a competitor of NO to the binding side of the non-heme iron between Q_A and Q_B.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Morphological and physiological characterisation and multigene phylogeny of the zygomycetous fungal order Mortierellales

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Mortierellales is one of the largest groups of Zygomycota. These fungi have practical importance as producers of polyunsaturated fatty acids, such as arachidonic acid, and as biotransforming agents of different organic compounds used in the pharmacological and chemical industries. Understanding the evolution of Mortierellales including the origin and evolutionary role of their enzymes may increase the biotechnological relevance of these fungi. Originally, this group was considered as a family of the order Mucorales. In the recent past, phylogeny of Zygomycetes had been analysed in detail revealing the need of the establishment of the new order Mortierellales. However, these works focused mainly on the Mucorales and the phylogenetic relationships within the Mortierellales remained unresolved. Molecular phylogenetic data suggest that the presently accepted, morphology-based taxonomy of the order is highly unnatural.

We inferred phylogenetic relationships of the Mortierellales using a combined matrix of nrITS, 5.8S, nrLSU, nrSSU, EF1-alpha and RPB1 sequences from 106 strains of *Mortierella*- and related taxa. PCR was carried out and the questioned sequences defined. Sequences were aligned by using the softwares MUSCLE and Probalign. The phylogenetic analyses were made by using Maximum Likelihood and Bayesian estimation. Phylogenetic trees were calculated on the strength of the sequences of each ribosomal subunit on its own and on a fourth, combined, large alignment including all of them. Results suggest that the genus *Mortierella* is paraphyletic and includes the genera *Gamsiella, Dissophora* and *Lobosporangium.* The relationships between the larger groups of the genus *Mortierella* also became clearer, as we found that *M. verticillata* and *M. humilis* are closely related to each other. The same conclusion goes to *M. gamsii* and *M. hyalina*. It seems that *G. multidivaricata* and *M. mutabilis* are in close relationship not only on a phylogenetic but also on morphological basis.

Morphological investigations were carried out using both light- and scanning electron microscopic techniques. Our goal was to reveal the fine structure of the observed characters, such as branching of the sporangiophores, ornaments of the sporangia and the mycelial structure. We found that phenotypic traits of these fungi strongly depend on the culturing conditions. We also investigated the carbon source utilization patterns of the fungi by using 67 different carbon sources. This research showed that delimitation of the species is difficult by using only morphological and/or physiological characters but merged with the phylogenetic results they improve the understanding of the relationships within this fungal group.

The research was supported by the international cooperation grant of DFG and OTKA (OTKA NN75255).

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Relationship between reactive oxygen (ROS) and reactive nitrogen species (RNS) and auxin in *Arabidopsis* development under copper excess

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Copper (Cu^{2+}) is an essential microelement but its excess influences the shoot and root architecture of plants. This heavy metal induces ROS production leading to oxidative stress condition. Moreover, alterations of nitric oxide (NO) levels can also be detected, which plays a role e.g. in cell death induction. Based on these, the aim of this study was to investigate the morphological and physiological responses and the possible relationship between ROS and RNS during short-term (7-day-long) and longer (17-day-old) copper exposure in the root tips of *Arabidopsis* using microscopic methods.

For the experiments (*Col-0*, WT), NO-overproducer (*nox1*), NO-deficient mutants (*nia1nia2*, *nia1nia2noa1-2*) and the S-nitrosoglutathione reductase (GSNOR)- deficient *gsnor1-3* plants were used. Also *Arabidopsis* plants with low (*vtc2-1* and *vtc2-3)* and high ascorbate content (*miox4*) were treated with 0, 5, 25 and 50 μM $CuSO_4$.

During short-term treatments, Cu^{2+} at a concentration of 50μM resulted in a large reduction in cotyledon area and hypocotyl and primary root lengths, accompanied by an increase in auxin levels. In cotyledons, a low Cu^{2+} concentration promoted NO accumulation, which was arrested by nitric oxide synthase or nitrate reductase inhibitors. The 5μM Cu^{2+}- induced NO synthesis was not detectable in *nia1nia2* or *nia1nia2noa1-2* plants. In roots, Cu^{2+} caused a decrease of the NO level, which was not associated with superoxide and peroxynitrite formation. Inhibition of auxin transport resulted in an increase in NO levels, while exogenous application of an NO donor reduced the auxin-dependent DR5::GUS expression. The elongation processes of *nox1* were not sensitive to Cu^{2+}, but NO-deficient plants showed diverse growth responses.

Copper excess caused the inhibition of stem and root growth of 17-day-old *Arabidopsis*, during which cell elongation, division and expansion were also modified. The symptoms of stress induced morphogenic response (SIMR) were found in the root system of 25 μM

Cu^{2+}- treated plants. In both organs, the decrease of auxin-dependent gene expression was found, which can partly explain the growth inhibitions.

In plant organs, Cu^{2+} treatment results in severe morphological responses during which the endogenous hormonal balance and signal transduction are affected. Auxin and NO negatively regulate each other's level and NO intensifies the metal-induced cotyledon expansion, but mitigates elongation processes under short-term Cu^{2+} exposure. Besides hormonal system, nitric oxide metabolism was also influenced by copper. In the root tips, this heavy metal excess induced NO generation, while NO content in lateral roots was not affected by the treatments. Using *nia1nia2* mutants, nitrate reductase enzyme as a putative source of Cu^{2+}-induced NO was identified in *Arabidopsis* primary roots.

Moreover, ROS levels were also influenced by copper. Under copper treatment, NO might play a protective role by regulating ROS levels possibly through modulation of the antioxidant activity.

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Studies on the cellular functions of newly discovered Prion family protein Shadoo

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The SPRN gene encodes the Shadoo glycoprotein (Sho), a central nervous system-expressed member of the prion protein super family. Sho is highly conserved from fish to mammals. SPRN is conserved in mammals, as is the prion gene PRNP, but in sheep SPRN and PRNP are both marked by polymorphic variation, suggestive of a shared selection pressure within these scrapie disease-prone livestock animals. In rodent models of prion disease there are reduced levels of Sho in infected tissues, defining a form of cross-regulation between full-length Sho holoprotein and PrP^{Sc}. The similarities between Sho and PrP N-terminus are the natively unfolded nature of polypeptide chains, a hydrophobic domain and tandem repeats with positively charged residues. Indeed, scrutiny of Sho's biochemical properties in uninfected cells has revealed overlaps with the properties of PrP^{C}, these including shared protein binding partners.

Prion protein functions as a metal binding protein because divalent cations such as copper, zinc and manganese can bind to the octapeptide repeat sequences in the N-terminus of PrP^{C}. Since the binding of these metals to the octapeptide has been proposed to influence both structural and functional properties of prion protein, alterations in transition metal levels can alter the course of the disease. As a member of the prion protein super family, we thought that Sho protein may behave like PrP as a metal binding protein, although it lacks the octapeptide region.

We carried out experiments on N2a cell lines stably expressing the Sho protein applying various concentrations of transition divalent metals. We could see the membrane internalisation of Sho protein induced by Co^{+2}, Mn^{+2} and Zn^{+2} ions. Also, we observed that the Sho expressing cells showed protection against the cytotoxic effects of Mn^{+2}.

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Identification and characterization of a novel circadian clock mutant in *Arabidopsis thaliana*

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

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

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In order to identify novel components of the circadian system in *Arabidopsis thaliana*, we carried out a large-scale forward genetic screen. Several mutants, displaying altered rhythmic expression pattern of the *CAB2:LUC* reporter gene in continuous red light conditions, were isolated. The mutant *ct12* (*circadian time 12*) showed about 2 h period shortening under the screening conditions and was selected for further analysis. The mutation affected the expression of several clock-controlled genes in the same manner and the short period phenotype was independent of the light conditions. These findings indicated that the function of the core oscillator was altered in the mutant. In fact, expression of the core clock genes showed the expected short period phenotype, but the level of their expression was not affected significantly. This suggests that CT12 does not affect transcription of clock components directly. Consistent with the basic circadian dysfunction, *ct12* showed early flowering phenotype in short day conditions. We have provided experimental evidences that the flowering phenotype of the mutant is caused by the altered circadian period/phase. Moreover, *ct12* mutants produce long hypocotyls in red but not in blue light, suggesting a positive role for CT12 in light responses mediated by the red/far-red light absorbing phytochrome photoreceptors.

Genetic mapping followed by transgenic complementation showed that the *CT12* gene encodes a putative acyl-transferase. Although the exact biochemical function of this protein and the way it affects the function of the clock remains to be elucidated, we hypothesize that CT12 could represent a link between the clock and certain metabolic processes.

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The effect of recreational physical exercise on inflammatory markers in a rat model of colitis

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The sedentary lifestyle can lead to health problems such as metabolic syndrome including obesity with hypertension, insulin resistance and high blood lipid levels. Metabolic syndrome is associated with a chronic low-grade inflammatory state and oxidative stress. Many studies reported that physical activity is an effective way of controlling body weight, but the influence of long term low intensity exercise on inflammation and activity of anti- and proinflammatoric enzymes is not well known. Heme oxygenase-1 (HO-1), which is the inducible isoform of heme oxygenase enzyme (HO), is thought to play an important role in the protection of tissues from oxidative injuries. Another enzyme involved in oxidative stress and inflammation is nitric monoxide synthase enzyme (NOS) with 3 isoforms: the inducible (iNOS) and the two constitutively expressed (cNOS) isoforms namely neuronal NOS (nNOS) and endothelial NOS (eNOS). Nitric monoxide (NO) produced in different amount by the three NOS isoforms can be both harmful and beneficial. We used a rat model, trinitrobenzene-sulphonic acid (TNBS) induced colitis, to investigate the changes of inflammation and activity of HO and NOS enzymes in the colon after running.

We investigated the effects of long-term leisure-type physical exercise on the activity of HO, NOS and myeloperoxidase (MPO, an inflammatory marker) enzymes in the trinitrobenzene-sulphonic acid (TNBS) induced colitis in rats in dependence on time.

After 3, and 6 weeks self-administered physical activity (running wheel) male Wistar rats were treated with TNBS (10 mg). After 72 h TNBS challenge we measured colonic inflammatory parameters and HO, iNOS, cNOS, MPO activity.

While after 3-week running we found no difference in the severity and extent of colonic inflammation in the sedentary and running TNBS treated group, the 6-week freewheel running significantly increased the activity of HO (from 1,3±0,2 to 2,8±0,3 nmol bilirubin/h/mg protein), constitutive NOS isoforms (from 321,1±35,2 to 438,0±30,1 pmol/min/mg protein). The TNBS challenge after 6 weeks running significantly decreased the level of inflammatory markers including extent of lesions (from 54,6±2,6% to 42,9±3,2%), severity of mucosal damage (from 7,6±0,3 to 6,6±0,3) and the level of MPO activity (from 880,6±79,3 to 568,4±59,9 mU/mg protein), increased the activity of cNOS (from 108,9±25,6 to 333,9±32,3 pmol/min/mg protein) decreased the iNOS activity (from 217,6±26,4 to 128,9±15,8 pmol/min/mg protein), but did not changed the activity of HO compared to the sedentary TNBS-treated group.

Long lasting recreational physical activity, at least 6 weeks by rats, improves body's defence mechanisms. Physical activity-induced increasing activation of HO and cNOS systems, decreased activation of iNOS system may play role of these mechanisms including colonic inflammation.

This work is supported by the TÁMOP 4.2.1./B-09-1/KNOV-210-0005 and TÁMOP 4.2.2.-08/1-2008-0006 research grants.

The presentation is supported by the European Union and co-funded by the European Social Fund.

Project title: "Broadening the knowledge base and supporting the long term professional sustainability of the Research University Centre of Excellence at the University of Szeged by ensuring the rising generation of excellent scientists."

Project number: TÁMOP-4.2.2/B-10/1-2010-0012.

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Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Connection between sulfur metabolism and Hyn hydrogenase in *Thiocapsa roseopersicina* BBS

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The purple sulfur phototrophic bacterium, *Thiocapsa roseopersicina* BBS preferably utilizes sulfur compounds as electron donors and carbonate as inorganic carbon source for growth. Its sulfur metabolism is similar to that of *Allochromatium vinosum* but several variances could be recognized. *T. roseopersicina* contains several enzymatic routes for oxidation sulfur compounds, such as the modified Sox cycle which has an indispensable role in the assimilation thiosulfate, few sulfide oxidoreductases and the Dsr complex which is likely linked to sulfur oxidation. All these processes release electrons which – in principle - might be converted to H_2 via the hydrogenases and/or nitrogenase of the strain.

Hydrogenases are metalloenzymes capable of oxidation of molecular hydrogen and proton reduction. *Thiocapsa roseopersicina* BBS has four active NiFe hydrogenases. Hox1, Hox2 are cytoplasmic NAD^+ reducing hydrogenases, while the other two enzymes (Hyn, Hup) are bound to the membrane. The Hup and Hox1 hydrogenases are likely connected to the central quinone pool.

The main electron transport routes to/from the hydrogenases are not fully understood. In order to disclose these metabolic pathways, we cultivated single hydrogenase containing strains, in the presence of various kind of electron donors and the amounts of H_2 and various sulfur compounds were followed.

Hyn hydrogenase can produce hydrogen in the absence of carbonate. Under these conditions, sodium thiosulfate could promote hydrogen evolution, while the expression level of Hyn remained the same. Therefore, the elevated H_2 might be derived from the more intense metabolic flux. It was also shown that the oxidation of zero-valent sulfur can donate electrons to Hyn. Under these conditions, sulfur is an exclusive electron donor for both hydrogen evolution of Hyn and hydrogen sulfide formation which are consequently competitive processes. These results suggest that Hyn hydrogenase has a role in the elimination of extra electrons released from sulfur oxidation and protection against toxic effect of sulfide. Hydrogen evolution of Hyn hydrogenase was found only under illumination Moreover, the oxidation of various sulfur compounds was also blocked in darkness, therefore the light dependency of hydrogen evolution might be an indirect consequence of the light requirement of sulfur oxidation.

Glutathione amide forms were shown to be potential redox carrier in purple sulfur bacteria. Their role was investigated in the electron transport between sulfur metabolism and Hyn hydrogenase. In the absence of glutathione amide reductase there was an elevated hydrogen evolution by Hyn which indicated a competition between glutathione amide and Hyn hydrogenase for the electrons.

Oppositely, in the presence of elemental sulfur, hydrogen addition increased the Hyn mediated hydrogen sulfide formation, thus the connection between Hyn hydrogenase and sulfur metabolism was proved to be bidirectional. The Hyn dependent hydrogen sulfide formation was not light dependent. It was also pointed out that the two electron transport subunits of HynSL -Isp12- were indispensable in this linkage.

The interrelationship of hydrogen and sulfur metabolism was clearly demonstrated at physiological level. Based on these results, an integrated – but still hypothetical – electron transport model was outlined.

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The supramolecular organization of photosystem II in vivo studied by circular dichroism spectroscopy

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The light reactions of photosynthesis in higher plants take place in granal chloroplast thylakoid membranes, which contain chirally organized macrodomains composed of photosystem II (PSII) supercomplexes associated with light harvesting antenna complexes (LHCIIs). The physiological relevance of this hierarchic organization, which often manifest itself in semicrystalline macro-assemblies, has not been elucidated but the diversity of the supramolecular structures and their reorganizations under different conditions indicates its regulatory role. The present work focuses on the structural and functional roles of different components of LHCII-PSII supercomplexes. We used various growth conditions, influencing the protein composition, and different Arabidopsis mutants (koCP24, koCP26, koPsbW, koPsbX, dgd1), with altered organization of the membranes, and measured their circular dichroism (CD) spectra as well as their chlorophyll fluorescence kinetics to characterize the chiral macro-organization of the chromophores and the functional parameters of the membranes, respectively.

We have shown that the LHCII components play important roles in the macro-organisation of thylakoid membranes. We found that although these pigment-protein complexes themselves have only limited capacity to form ordered arrays in vivo, they can promote the

Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
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formation of long-range chiral order of chlorophyll and carotenoid molecules, as manifested in Ψ-type CD. The role of LHCII in determining the CD is mainly exerted via organising the PSII supercomplexes into chirally organized macro-assemblies, rather than via increasing the number of interacting chromophores. PSII associated with LHCII appears to possess higher ability to form macro-domains than either the core complexes or LHCIIs on their own. Coexistence of the LHCII and PSII core in the membrane - but without coupling (by LMW proteins) - appears to result in less ordered arrays.

Our data also reveal specific functions of some of the protein and lipid compounds in the light adaptation processes of plants.

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Role of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) in the trigeminovascular system

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Abnormal activation of trigeminovascular system (TS) plays important role in the development of migraine, but its precise mechanism is still unknown. Recent clinical and experimental data have suggested that Pituitary Adenylate Cyclase-Activating Polypeptide-38 (PACAP-38) might contribute to the evolution of migraine-attacks. Therefore, we aimed to examine the PACAP-immunoreactivity (IR) in blood plasma of migraineurs, in interictal and ictal periods as well as in blood plasma and nerve tissues in two animal models of activated TS.

Adult Sprague-Dawley rats were got involved in the experiments. Electrical stimulation (ES) of the trigeminal ganglion (TRIG) was applied at 10 Hz, 1 mA for 30 min and in a separate group of rats 10 mg/kg dose of nitroglycerol (NTG) was injected ip. Peripheral blood samples were collected, and three brain regions, involved in migraine (caudal trigeminal nucleus-TNC; TRIG; cervical 3-5 of the spinal cord-SC) were dissected at different time-points after the stimulation of the TS.

In the clinical study 40 control subjects and 60 migraineurs were examined, selected by the criteria of the International Headache Society. Blood samples were collected from 21 migraineurs in the interictal and drug-free ictal periods.

In both case, the blood samples were taken to tubes containing EDTA and protease inhibitor, then the plasma was separated (2000 rpm 10 min 4°C). The plasma and nerve tissue samples were stored at -80°C till the PACAP radioimmunoassay measurements.

In rats the plasma PACAP-IR significantly increased 90 and 180 min after ES compared to the sham-stimulated and intact control groups. ES also evoked a remarkable elevation of PACAP level in the TNC at the 180 min time-point. In the NTG-model plasma PACAP-IR remained unchanged, but significant PACAP-IR increase was observed in the TNC 90 and 180 min following the chemical stimulation. The level of this peptide was not substantially altered in the TRIG and the SC in either model.

Significantly lower PACAP-38-IR were detected in human interictal migraine samples, than in the control group (Student's *t*-test for unpaired comparisons; $p<0,002$). Self-control comparison of PACAP-38-IR of 17 migraineurs in the ictal and interictal periods showed significant elevation during the attack (Student's *t*-test for paired comparisons; $p<0,002$).

It is concluded, that in animals the elevated PACAP-IR in the systemic circulation and/or in the TNC induced by PACAP release from both the peripheral and central terminals of the trigeminal pseudounipolar neurones.

The reduced concentration of PACAP-38 in the interictal period might be due to energy deficit. After a trigger the peptide can release from the sensory nerve terminals. The level of PACAP starts to significantly increase in the systemic circulation and induces vasodilatation, neuronal activation, sensitization, which are responsible for the initiation of pathomechanism of primary headache, like migraine.

The crucial role of PACAP in the activation mechanisms of TS is assumed. The nervous system specific examinations of PACAP can provide new perspectives to identify a new target in the therapy of migraine.

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