Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARIES

Distribution of two molecules: KCC2, which plays a role in the postsynaptic responses evoked through $GABA_A$ receptors, and δ-subunit-containing $GABA_A$ receptors, which appoint neruogliaform cells in the neuronal network that can uniquely create the long-lasting postsynaptic $GABA_A$– and $GABA_B$ receptor mediated inhibition on target neurons

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The neuron-specific potassium-chloride cotransporter 2 (KCC2) plays a crucial role in adjusting intracellular Cl^- concentrations. The lack of KCC2 in the plasma membrane of the axon initial segment (AIS) of pyramidal cells contributes to variable reversal potentials for perisomatic γ-aminobutyric acid $GABA_A$ receptor-mediated postsynaptic potentials, but the distribution of KCC2 in pyramidal dendrites remains to be established. We applied high-resolution pre-embedding immunolocalization to quantify KCC2 concentrations along dendritic, somatic and axonal regions of rat hippocampal principal cells. Confirming our results on neocortical pyramidal cells, membranes of AIS of CA1 pyramidal cells and dentate granule cells contained 6.4 ± 11.9% and 6.6 ± 14.1% of somatic KCC2 concentrations, respectively. Concentrations of KCC2 in basal dendritic shafts of stratum (str.) oriens were similar to somatic levels (109.2 ± 48.8%). Along apical dendritic shafts of CA1 pyramidal cells, the concentration of KCC2 showed a complex profile: normalized to somatic levels, the density of KCC2 was 124.5 ± 15.7%, 79 ± 12.4% and 98.2 ± 33.5% in the proximal and distal part of str. radiatum and in str. lacunosum moleculare, respectively. Dendritic spines of CA1 receiving excitatory inputs contained 39.9 ± 8.5% of KCC2 concentration measured in shafts of the same dendritic segments targeted by GABAergic inputs. Dendrites of dentate granule cells, the other glutamatergic cell type in hippocampus, showed higher KCC2 concentration compared with the soma (148.9 ± 54%), but no concentration gradient was detected between proximal and distal dendrites. In conclusion, the density of KCC2 in hippocampal principal cells increases along the axo-somato-dendritic axis with cell type-specific distribution profiles within the dendritic tree.

For the localization of delta subunit of $GABA_A$ receptors ($GABA_{AD}$) we used immunofluorescent method on the neocortex. In addition to a weaker neuropil labeling in supragranular layers presumably due to dendrites of pyramidal cells, a subset of interneurons was strongly positive for the $GABA_{AD}$ The identity of strongly $GABA_{AD}$immunopositive interneurons was initially tested by multiple immunoreactions showing that α-actinin2, known to be expressed by electrophysiologically identified neocortical neurogliaform cells, were present in 65±12 % of $GABA_{AD}$receptor containing cells. However, no overlap was found with interneuron markers such as parvalbumin, somatostatin, calbindin, calretinin and vasoactive intestinal polypeptide. In addition, immunocytochemical labeling patterns in the hippocampus were in line with earlier electrophysiological data showing relatively large tonic inhibition in hippocampal granule cells and molecular layer interneurons in the dentate gyrus and weaker currents in pyramidal cells of the hippocampus. In the CA1 and CA3 regions, a subset of interneurons at the border of stratum radiatum and lacunosum moleculare and another population of interneurons close to stratum pyramidale show strong immunolabeling for $GABA_{AD}$receptors. These interneurons presumably correspond to hippocampal neurogliafrom cells and ivy cells, respectively.

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The molecular and metabolic connections of Hox1 hydrogenase in *Thiocapsa roseopersicina*

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Hydrogen is considered as the most promising fuel of the future, since it can be produced and oxidized without emission of green house gases. Biological systems offer easily reproducible biocatalysts for production of hydrogen. Several microorganisms, including both phototrophs and non-photosynthetic heterotrophs are able to metabolize molecular hydrogen by means of their hydrogenase or nitrogenase enzymes.

Our model organism, *Thiocapsa roseopersicina* is a Gram-negative, anaerob purple sulphur photosynthetic bacterium capable to produce molecular hydrogen as a byproduct during photochemolitoautotrophic growth. The strain can grow on inorganic substrates and utilize small organic molecules such as volatile acids and glucose. It contains four NiFe hydrogenases: two of them are soluble (Hox1 and Hox2) while the other two enzymes are attached to the membrane. The strain has complex sulphur metabolism it is able to fix molecular nitrogen and

to accumulate various storage materials. The deposition of storage compounds is a widespread strategy among microbes to survive when the nutrient or energy sources are depleted.

My study focuses on the cytoplasmic Hox1 hydrogenase (Rákhely et al. 2004) which is able to reduce protons and oxidize hydrogen *in vivo* depending on the redox status of the cell and environment. The enzyme consits of five subunits: Hox1Y and Hox1H are the small and large hydrogenase subunits, respectively; Hox1F and U are the diaphorase subunits, while Hox1E subunit is involved in the electron transport. The aim of this work is to clarify the physiological contexts of Hox1 hydrogenase; *i.e.* to examine its molecular/metabolic/redox connection to the storage materials and the bioenergetic membrane.

The Hox1 hydrogenase can produce hydrogen under illumination and in the dark. The hydrogen production requires excess electrons derived from e.g. thiosulfate under continuous illumination. On the basis of experimental and *in silico* data, it is hypothesized that the Hox1 hydrogenase is connected directly to photosynthetic membrane via the membrane-located NADH-ubiquinone oxidoreductase complex. The Hox1EFU subunits have remarkable sequence similarity to the NuoEFG subunits which are dissociable from the membrane and they have NAD^+ -reducing activity. According to our model, the Hox1EFU subunits can replace the NuoEFG subunits allowing Hox1 to function as a valve. When the central quinone pool is overreduced, excess electrons can be removed in the form of hydrogen by means of Hox1 hydrogenase, otherwise NADH is produced. In order to prove this model, a proteomic apporoach was chosen and affinity chromatography was used to identify interacting protein partners.

Hydrogen can also be produced in the dark through the Hox1 hydrogenase. In this case, the excess of electrons is supposed to arise from stored materials accumulated during photosynthetic growth. Depending on the nutrient supply during growth, *T. roseopersicina* can accumulate elementar sulphur, polyphosphate poly(3-hydroxyalkanoates) and glycogen. A systematic investigation of the physiology and hydrogen production of the cells indicated glycogen as a potential source of electrons for the Hox1 hydrogenase in the dark. The genome of the strain has been sequenced and genes coding for proteins involved in both glycogen synthesis and catabolism were identified. In order to confirm this metabolic connection, both the glycogen synthesis and breakdown were disrupted by genetic tools and a comparison of the hydrogen production and glycogen content of the mutant and the control strains revealed a metabolic linkage between the glycogen and hydrogen metabolism.

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Diabetes-related structural, molecular and functional alterations in capillaries running in the vicinity of myenteric plexus in streptozotocin-induced diabetic rats

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It has recently been demonstrated that the nitrergic subpopulation of myenteric neurons is especially susceptible to developing neurodegenerative damage in diabetes. The nitrergic neurons located in different gut segments had different susceptibilities to diabetes. Their different responsiveness to insulin treatment had also been revealed, which suggest that the neuronal microenvironment is critical to evolving diabetic nitrergic neuropathy. Although the relationship between the presence of enteric neuropathy and impaired gastrointestinal motility in humans and also in rodent models are well documented, the impact of diabetes on capillaries within the intestinal wall has been completely overlooked until now.

Since the myenteric ganglia are not vascularizated, accordingly the mesenterial capillaries adjacent to the myenteric plexus play a key role to supply them. Therefore we supposed that diabetes-associated alterations, which influence the permeability of these capillaries, may be critical to developing enteric neuropathy observed in streptozotocin-induced diabetics. The diabetes-related endothelial dysfunction leads to decreased bioavailability of endothelial cell-derived nitric oxide and at the same time to increased amount of toxic free radicals before the clinical symptoms appear.

Therefore, the primary question of our study was whether diabetes influences the structural, molecular and functional properties of capillary endothelium closely related to the myenteric plexus.

Ten weeks after the onset of diabetes, different gut segments of control, streptozotocin-induced diabetic and insulin-treated diabetic rats were processed for electronmicroscopic and molecular studies. The thickness of basement membrane (BM) surrounding blood vessels and the size of the individual caveolar compartments were measured by electronmicroscopic morphometry. The quantitative features of blood-tissue exchange of endogenous albumin were investigated by postembedding immunohistochemistry. The quantitative changes in the expression of endothelial nitric oxide synthase (eNOS) and its negative regulatory protein, Caveolin-1 (CAV-1) were elucidated by postembedding immunohistochemistry, RT-PCR technique and western-blot analysis in the endothelium of microvessels around myenteric plexus.

Although the differences between the intestinal segments are well pronounced, region-specific thickening of BM and enlargement of caveolar compartments was demonstrated in diabetic animals. The amount of serum albumin taken up by the plasmalemmal vesicles

and transported to the interstitium was enhanced in diabetics compared to controls. The overexpression of CAV-1 and eNOS was also documented in diabetic groups suggesting enhanced transendothelial transport and hyperpermeability of these capillaries. In some cases immediate insulin replacement prevented the development of diabetes-related region-specific alterations.

These results indicate a close relationship between the segment-specific diabetic nitrergic neuropathy and vascular dysfunction of mesenterial capillaries running in the vicinity of myenteric plexus in the gut. Our data provide morphological, functional and molecular evidence that the endothelial cells of these vessels are direct targets of diabetic damage. We suggest therefore that these endothelial cells are potential therapeutic targets to prevent the development of the nitrergic neuropathy and the gut motility disorders in diabetic patients.

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Molecular characterization of the computationally predicted *miR-282* microRNA gene of *Drosophila melanogaster*

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MicroRNAs have been discovered as a new type of regulatory genes whose transcripts are marked by a representative intermedier form, the hairpin structure. Due to this typical secondary structure and the advanced bioinformatic methods, hundreds of new miRNA genes have been identified in animals, plants and even viruses. Hundreds of target genes for every single miRNA also have been predicted. In this way, a huge amount of data has been generated, which is waiting for interpretation and experimental confirmation.

MicroRNAs (miRNAs) are ~22 nucleotide long, single-stranded regulatory RNAs that bind to complementary sequences in the three prime untranslated regions of target mRNAs thereby, negatively regulating (by transcript degradation and translational suppression) the target genes. Although a significant group of miRNA genes is found in the introns or sometimes in exons of protein and non-protein coding genes, most microRNA genes lie in intergenic regions and contain their own promoter and regulatory components. MicroRNA primary transcripts (pri-miRNAs) are synthesized by RNA polymerase II. In this way, pri-miRNAs which range couple thousands of nucleotides in length have 5' m7G cap structure and usually subjected to polyadenylation in their 3' end. However the functional analyses are still in their infancy because they are hampered primarily by redundancy among miRNA genes occurring when different miRNAs share the same 5' seed sequence or their target(s) and if they are coexpressed. Moreover, most miRNA mutants show subtle or low-penetrance defects that may be difficult to identify. As a consequence, in only few cases can lead the lack of miRNA function to robust phenotypes. Despite of these findings, it has become clear today that miRNAs are required for the fine tuning of the regulation of sometimes very complex mechanisms and participate in the regulation of almost every biological processes investigated so far.

While in the fruit fly (*Drosophila melanogaster*) 176 miRNAs has been computationally predicted to date (miRBase release 16), the real target mRNAs and biological function have been assigned to only a dozen of them. We characterized a miRNA gene, *mir-282* of *Drosophila melanogaster* which is evolutionary conserved among insects. The *mir-282* gene is located on the third chromosome within a 13.9 kb genomic region devoid of any protein coding genes and our data strongly suggest an independent *mir-282* gene whose primary transcript has a distinct 5' start with a CAP and a few alternative 3' ends with polyA tail. We have determined the correct size of the pre- and mature *mir-282*. We found that the *mir-282* locus encodes a functional transcript which influences viability, longevity and egg production in Drosophila, most likely through the regulation of cAMP level at pupal stage.

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Neuroprotection with novel KYNA-amide

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Acute protection and the recovery of neurons from cerebral ischemic insults of whatever nature give rise to the main drive in the development of neuroprotective strategies.

The most widely accepted concept relating to ischemic brain damage is the concept of excitotoxicty.

Treatment with N-methyl-D-aspartate receptor antagonists is a widely accepted method with which to stop the advance of excitotoxic processes and concomitant neuronal death. From a clinical aspect, competitive glycine- and polyamine-site antagonists with relatively low affinity and moderate side-effects are taken into account. Endogenous kynurenic acid (KYNA) acts as an antagonist on the obligatory co-

agonist glycine site, and has long been at the focus of neuroprotective trials. Unfortunately, KYNA is barely able to cross the blood-brain barrier. Accordingly, the development and synthesis of KYNA analogs which can readily cross the BBB have been at the focus of research interest with the aim of neuroprotection.

A novel KYNA analog, 2-(2-N,N-dimethylaminoethylamine-1-carbonyl)-1H-quinolin-4-one hydrochloride (Patent Application No: 104448-1998/Ky/me), recently proved to be neuroactive in several experimental paradigms. The analog effectively reduced c-fos and nNOS activation in an experimental animal model of migraine, effects interpreted as due to NMDA blockade. Moreover, in an *in vitro* comparative electrophysiological study, this compound was found to have the same neuromodulatory attributes as KYNA. NMDA antagonism was also acknowledged. 1 mmol of the analog administered i.p. effectively reduces the amplitudes of hippocampal population spikes. Regarding these properties, we estimated the neuroprotective capability of a novel kynurenic acid analog in transient global forebrain ischemia, measuring the rate of hippocampal CA1 pyramidal cell loss and the preservation of long-term potentiation at Schaffer collateral-CA1 synapses.

The neuroprotective potential was reflected by a significantly diminished hippocampal CA1 cell loss and preserved long-term potentiation expression. The neuroprotective effect was robust in the event of pretreatment, and also when the drug was administered at the time of reperfusion.

A detailed analysis of the behavioral effects of this new compound appeared to be extremely important, and we have therefore investigated it from several aspects.

In a preliminary investigation of the effects of the analog on mice, we performed open-field tests of the locomotor activity and exploratory drive. The influence of the analog on spatial orientation and learning was also assessed in the radial arm maze imprinting test. In the Morris water maze tests we examined its effects on the working memory and long-lasting reference memory of rats.

It emerged that there is a dose of this KYNA-amide which is neuroprotective, but does not worsen the cognitive function of the brain. This result is significant in that a putative neuroprotectant without adverse cognitive side-effects is of great benefit.

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Developmental regulation of brassinosteroid distribution in *Arabidopsis*

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Brassinosteroids (BRs; steroidal phytohormones) are essential regulators of plant growth and development. Unlike most other hormones, BRs are not subject to active transport, but exert their effects locally, in a paracrine manner. Local BR levels are efficiently controlled by the coordinated actions of biosynthetic and degradative gene functions, which ensure both homeostatic and differential regulation. While the transcriptional regulation of BR biosynthetic genes is known in great detail, its direct effects on the hormone production and accumulation are still to be clarified.

The aim of our study is to find out how castasterone and brassionolide, the two biologically active forms of BRs, are distributed in the model plant *Arabidopsis thaliana*. To observe developmental changes in the hormone accumulation, we generated transgenic plants expressing reporter genes under the control of an artificial BR-responsive promoter. The BR response constructs will be used for monitoring developmental BR adjustments during morphogenic events, such as germination and the differentiation of reproductive organs. Parallelly, we determine the bioactive BRs in all *Arabidopsis* organs via CG-MS analyses, in order to construct a comprehensive map of hormone distribution in the adult plant. In another approach, we initiated studies on the role of regulated hormone distribution during embryonic development. This line of research utilizes GFP and LUC reporter-tagged versions of the CYP85A2 enzyme that catalyzes the rate-limiting step of BR biosynthesis. The transgenic lines expressing these chimeric proteins will be helpful in elucidating the induction and spatial pattern of embryonic BR synthesis, and its correlation with the developmental auxin re-distribution that has been well characterized.

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The examination of the telomer protecting *Drosophila melanogaster* gene (*dtl*)

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In *Drosophila melanogaster* chromosome ends consist of retrotransposon arrays, the well-defined, short telomeric repeats, characteristic of human and other telomerase-containing organisms are absent. Consequently, in Drosophila there is no need for the sequence-specific,

repeat-recognising telomere capping complex, called shelterin. Instead a new complex, named terminin has evolved in *Drosophila melanogaster* and closely related species, which has an essential role in telomere capping and maintenance. Genes encoding proteins involved in this complex, appeared recently in the phylogenesis and since then these genes have been quickly changing. The proteins are functional analogues of the members of shelterin complex. Terminin complex (just like shelterin) interacts with proteins involved in the DNA damage signaling pathways. The lack of either capping proteins causes telomere disruption, therefore activates DNA damage checkpoints and fuses the end of the chromosomes.

The bicistronic *Drosophila* gene *dtl/tgs1* (*CG31241*) encodes two proteins; *Drosophila Tat-Like/Drosophila Telomere Lost* (DTL) – is a member of the telomer protecting terminin complex, and *Trimethyl-Guanin Synthase* (TGS1) that catalyzes the trimethyl-guanine (TMG) cap synthesis of sn- and snoRNAs (Komonyi et al 2005. and 2009). Our data indicate, that both products of *dtl/tgs1* gene are essential. Mutations which affected *dtl* (but didn't have any effect on *tgs1)* caused telomere associations (TAs), whereas the absence of TGS1 (but not DTL), resulted defected TMG cap containing RNA synthesis. However, DTL and TGS1 seem to have distinct functions such as telomere maintenance and RNA processing, these two functions might possibly be interconnected as TMG cap containing RNAs play an important role in telomere maintenance.

We payed particular attention to determine the role of the two proteins in the germ line. In the lack of maternal *dtl/tgs1* product, the development of eggs is significantly abnormal. After fertilisation, erratic cleavage division can be observed and only a few embryos reach the zygotic expression stage. For better understanding, we have investigated the unique effect of either the DTL or the TGS1 protein during oogenesis.

Since DTL is a putative member of terminin complex, we have supposed that it interacts with proteins involved in telomere maintenance. Therefore, we have performed yeast two hybrid experiments to find possible interacting partners. We have overexpressed FLAG epitop-labelled DTL in transgenic flyes in order to purify DTL containing complex and other interacting partners.

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The role of Rac1 in stress signaling

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The heat shock response (HSR), one of the most studied cellular homeostatic mechanisms, is involved in the maintenance of cell functionality during stress. Yet, the whole stress signaling pathway has not been elucidated. In line with the membrane thermosensor model, mild stress, or "membrane defects" caused by different disease states, is sensed by changes in the fluidity and microdomain structure of membranes, influencing membrane localized signaling activities. In favor of this model, our group exposed different mammalian cells to various membrane fluidizers or drugs with the ability to interact with certain membrane lipids and found substantial modulation of heat shock protein (Hsp) expression. One possible signaling pathway originating from plasma membrane involves the lipid kinase, PI3kinase, which in turn activates the small GTPase, Rac1. Through downstream signaling cascade to MAP kinases, the main transcription factor, HSF1 is activated leading to Hsp synthesis. It was shown that Rac1 translocation to the plasma membrane is essential for activating downstream effectors and its membrane binding is determined at least in part by membrane lipids. In favor of "membrane stress sensor" model, our working hypothesis was that Rac1 pathway is involved in stress signaling through the effect of stress on membrane microdomain organization.

To study Rac1 involvement in HSR, we created tetracycline inducible stable mutant B16-F10 cells which are either dominant negative or constitutively active for Rac1. Though the antibiotic resistance showed stable clones, Rac1 protein expression was not detectable after induction. Now we use the "Sleeping Beauty Transposon" for stable Rac1 gene transfer in B16F-10cells.

As a proof of concept we have shown that using specific Rac1 inhibitor, NSC23766 and Capsaicin, the heat shock response decreased remarkably in dose dependent manner. Moreover, elevated levels of heat shock response achieved by addition of a Hsp co-inducer, BGP-15 is diminished by Rac1 inhibitors. We also documented that the amount of phosphorylated, active transcription factor, HSF1 decreased in inhibitor treated samples. In order to document the different localization of Rac1 in heat treated B16 cells we made immunofluorescence microscopy and Western blots on isolated membrane fractions.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Towards the understanding of genome-wide redistribution mechanisms of the RNA Polymerase II transcription machineries upon Ultraviolet B irradiation

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One of the main reasons of skin cancer is the Ultraviolet (UV) irradiation coming from the Sun. Depending on the wavelengths we distinguish UV-A (400-320nm), UV-B (320-280nm) and UV-C (280–100nm) radiation. Although UV-C has the most genotoxic effect on cells, this wavelength is fully absorbed by the Earth's atmosphere, thus UV-B is the most common source of skin cancer by inducing pyrimidine dimers (CPD) and (6-4) photoproducts on the DNA. Intrinsic (block of transcriptional machinery) and extrinsic (cell membrane damage) signals will trigger the p53 pathway, which will lead to cell cycle arrest and DNA repair or apoptosis in a dose dependent manner. The given response to UV irradiation depends not only on the dose but also on the wavelength.

Transcription in eukaryotes is a tightly regulated, multistep process. Gene specific transcriptional activators, several different coactivators and general transcription factors are necessary to access specific loci to allow precise initiation of RNA polymerase II (Pol II) transcription. Upon different stress stimuli histone acetyltransferase (HAT) coactivator complexes play a crucial role in the maintenance of the eukaryotic chromatin architecture, in the regulation of locus specific transcription and in the establishment of consequent gene expression pathways (Toth et al. 2010).

Our goal is to gain insight in the alteration of the transcriptional machinery upon sub-lethal doses of UV-B irradiation in a human breast cancer model system (MCF7 cells). To this end first, we analyzed the global redistribution of the Pol II enzyme on the genome. We assume, based on several published results that the detectable presence of Pol II on the genome is mostly reflecting ongoing transcription. In addition, we would like to understand also how two of the major HAT complexes (ATAC and SAGA) participate in the maintenance of the genome and/or in gene regulation.

After 50 J/m^2 UV-B treatment we detected massive accumulation of CPD photoproducts by slot blot combined with western blot analysis, indicating that pyrimidine-dimers were still present in the genome 6 hours after irradiation. We examined the mRNA level of two well-characterized UV damage-responsive genes (*CDKN1A, GADD45A*) as a control using RT-QPCR. The results are concordant with previous observations, and show a slight gene induction between 2 and 6 hours after UV treatment. When we performed Chromatin Immunoprecipitation (ChIP) coupled Q-PCR and ChIP coupled to high throughput sequencing (ChIP-seq) using an antibody raised against the N-terminal end of the largest subunit of the RNA Polymerase II, we found a great decrease of Pol II occupancy at a large majority of promoters genome-wide 5 hours after UV irradiation when compared to the control (Gyenis et al. 2010). This overall promoter clearance can be due to a transcriptional block triggered by the known transcription coupled repair mechanism. By using several elaborate bioinformatics tools (*i.e.* seqMiner, DREM, etc.) capable of handling and compare genome-wide datasets, we were able to sort genes into several clusters based on Pol II alteration upon UV treatment at the different time points. We found that there is a slight increase of Pol II enrichment on stress response genes and we detected a dramatic and constant loss of Pol II enrichment on Histone gene clusters (Krishanpal et al. in preparation).

Our further objectives are:

To understand the molecular mechanisms by which Pol II presence at promoters is decreased genome-wide following UV-B treatment.

To investigate the role of HAT coactivator complexes in the regulation of UV-stress induced genes genome-wide. Moreover, we would also like to expand our above-described experiments to a human keratinocyte cell line to understand how skin cells respond to UV-B.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

The connection between structure and function of electron-transfer subunits in Thiocapsa roseopersicina BBS

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Hydrogen can be considered as a potential renewable alternative fuel replacing fossil resources. Hydrogen gas can be produced by biological systems via hydrogenase or nitrogenase enzymes. Numerous phototrophic microbes are able to capture light energy and produce hydrogen. Hydrogenases are catalyzing the following simple reaction: $2H^+ + 2e^- \leftrightarrow H_2$. The cells dispose excess electrons through hydrogen production catalyzed by hydrogenases, while oxidation of molecular hydrogen mostly provides electrons for reductive and/or various energy conserving processes, such as respiration. [NiFe] hydrogenases consist of a large and a small subunit. The large subunit contains the binuclear metallocenter which is the active site of the enzyme. The small subunit is responsible for the electron transport between the active center and the surface of the enzyme.

Thiocapsa roseopersicina BBS is an anaerobic purple sulfur phototrophic bacterium isolated from the North Sea. It can grow on inorganic carbonate with reduced sulphur compounds (sulphide, thiosulphide or elementary sulphur) as electron donors, but it can also utilize organic compounds (e.g. sugar and acetate).

There are two membrane-bound (HynSL and HupSL) and two soluble (HoxEFUYH, HoxFUYH) [NiFe] hydrogenases in *T. roseopersicina*. HynSL shows extraordinary heat stability and it is resistant to oxygen inactivation. The arrangement of the structural genes coding for this enzyme differs from the organization of common hydrogenases because the genes of small and large subunits are interrupted by two ORFs: *isp1* and *isp2* (Rákhely et al. 1998).

In silico sequence analysis disclosed that Isp1 contains five transmembrane helices and a heme b binding motif, while Isp2 resembles the heterodisulfide reductases and contains Fe-S clusters. Therefore, they probably play an electron transfer role from/to the Hyn enzyme. Both proteins have been shown to be important for the function of the HynSL enzyme *in vivo* but neither of them is required for its expression or *in vitro* activity (Palágyi-Mészáros et al. 2009). In the *isp1,2* mutant strain, the *in vivo* H_2-producing activity of the the Hyn hydrogenase was completely lost while its *in vivo* H_2 uptake activity was dramatically decreased. The exact physiological role of the Isp proteins in the organism is still unknown.

The aim of my project is to disclose the physiologcial role of the Isp proteins and to get deeper insight into the molecular details of their function. In order to study the essential residues in these subunit, Isp1 and Isp2 proteins were (over)expressed in homologous host and purified via their His/Strep/Flag tags or by immunoprecipitation with Isp2 polyclonal antibody. The *in silico* analysis of Isp1 protein revealed 18 conserved amino acids in the primary sequence: four of them might have role in binding of the b-type heme, the function of the other 14 amino acids is still unknown. My aim was to examine the role of these amino acids in the function of Isp1 protein. Therefore, using a vector which contains the *hynS-isp1-isp2-hynL* operon of *T. roseopersicina,* each conserved amino acid was replaced by another one of distinct properties. The mutant genes were transferred back into the strain and the effect of the mutations in Isp1 was monitored via the activity of Hyn hydrogenase. Beside the histidines (His83, 96, 180 and 198) which are involved in the heme binding, other residues were also identified which are essential or important for the physiological function of the Isp1 protein.

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The morphogenic role of brassinosteroid perception in *Arabidopsis*

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Brassinosteroids (BRs) are steroidal phytohormones that control multiple essential functions during plant development. In *Arabidopsis thaliana*, bioactive BRs are perceived at the cell surface by the extracellular domain of the plasmamembrane-localized leucine-rich repeat receptor kinase (LRR-RK) BRI1. Upon binding the hormone, this receptor initiates a phosphorylation cascade, which results in the stabilization of the BRZ transcription factors that activate or repress BR-responsive genes. Binding of the hormone facilitates heterodimerization of BRI1 with its BAK1 co-receptor, another LRR-RK, and initiates transphosphorylational self activation of the receptor complex. BRI1 was proposed to be constitutively expressed and uniformly distributed within the plant, assuming that BR responses depend mainly on local

Volume 55(1):197-212, 2011
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levels of the hormone. Our observations, however, indicated BR effects are also influenced by differential hormone responsiveness.

The aim of our studies was to characterize the expression of BRI1 and find out if it can influence developmental and organ-specific changes in BR sensitivity. In order to determine the transcriptional activity, we generated transgenic plants carrying *BRI1* promoter-reporter gene fusions. In *BRI1* prom-*GUS* plants histochemical analysis of glucuronidase activity revealed close correlation between BR-dependent elongation and transgene activity. On the other hand, time-course measurements with *BRI1* prom-*LUC* lines showed strong induction of *BRI1* activity upon germination, and that the expression level was increased by dark, whereas decreased by light treatments. To test how differential BRI1 accumulation can influence morphogenic events, we prepared transgenic lines that express in bri1 mutant background BRI1::LUC fusions, with full receptor activity, under the control of various organ-specific promoters. We found that *BRI1::LUC* expression via the photosynthesis-associated *CAB3*, vascular *SUC2*, and procambial *ATHB8* promoters resulted in different types of partial complementation, which all resulted in disproportionate organ development. The observed expression patterns and morphogenic effects of *BRI1* expression strongly suggest a role for the receptor abundance in determining the intensities of local BR responses.

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Identification of Novel Regulatory Factors of Plant Stress Responses Using New Genetic Approaches

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Plants frequently encounter abiotic stress conditions, such as drought, soil salinity, unfavorable temperature, submergence or high light. These conditions severely limit plant growth, development and productivity; plants have developed various defense mechanisms to increase stress adaptation. Drought and salinity are regarded as the major environmental stresses primarily impose osmotic stress on plants. Still poorly understood how plant defense mechanisms is actually performed against salt and osmotic stresses. To dissect plant signaling pathways *Arabidopsis thaliana* is the supreme genetic model, however as a glycophyte its tolerance to salt stress is limited.

Thellungiella salsuginea (*halophila)* is a close halophyte relative of Arabidopsis, tolerates drought and salinity as well as extreme cold, accordingly has turned to be a model system in salt tolerance research (Bressan 2001). Thellungiella possesses many prosperous attributes of Arabidopsis, like short life cycle, self-pollination, small genome size (about 2X of Arabidopsis), and even its genetic transformation can be accomplished by simple floral dipping. In addition Thellungiella genes show 90-95% sequence identity to Arabidopsis.

We have developed new genetic technologies to identify novel regulatory factors controlling salt tolerance. Random cDNA libraries of Arabidopsis and Thellungiella have been cloned into the estradiol inducible pER8 plant expression vector (Zuo et al. 2001) The transformation competent cDNA library of Thellungiella has been introduced randomly into Arabidopsis plants, and 20,000 transformant seedlings have been screened for salt tolerance in the presence of estradiol. Fourteen estradiol dependent salt tolerant lines have been isolated, and the inserted cDNA clones of these lines have been cloned and sequenced, the corresponding Thellungiella genes were identified by Arabidopsis sequence homology search. Salt tolerance was confirmed by repeated germination and growth assays in 10 lines, and 2 lines have been selected for further characterization. The line TL1-2 expressed the cDNA of a Thellungiella putative translational initiation factor, the insertion of TL1-26 line contained the cDNA of a putative RNA-binding aminopeptidase.

Another approach has been devised to identify salt stress regulatory factors at cellular level. Arabidopsis cell culture has been transformed with the Arabidopsis cDNA library and the transformed cells have been selected on plant culture media supplemented with salt and estradiol. Four cell colonies have been selected with superior growth on selective medium. cDNA inserts of these calli have been cloned and identified by sequencing. One of these cDNA inserts encoded a novel heat shock factor, its overexpression could improve various abiotic stress tolerance of transgenic Arabidopsis plants.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Functional characterisation of AnHMGB-A and AnHMGB-B „high mobility group" proteins of *Aspergillus nidulans*

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Aspergillus nidulans is an important modell-organism, many metabolic and regulation pathways and the genome sequence of this organism is known. The two proteins of our interest are members of the „high mobility group B" protein family that are present in both lower and higher eukaryotes.These proteins affect the expression of various genes on chromatin. Due to their DNA and protein binding ability they have an effect on the stability of chromatin-remodelling and transcription-initation complexes on chromatin.

Our main goal was to explore the phsyological role of AnHMGB-A and AnHMGB-B proteins. We deleted the coding sequences of the two proteins from the genome and constructed single and double deleted mutants that were subjected to various experiments to compare their phenotype to the wild type (wt).

The mycelia of Δ*AnhmgB-A* strain showed decrased growth rate, abnormal shape of cell wall and altered osmotic tolerance in comparison to the wt. Abnormal distribution of reactive oxygen species within the mycelia (central location for mutant and peripheric for the wt) was observed by nitro-blue tetrazolium staining that could result the decreased growth rate and compact growing of the mutant. We observed decreased trehalose level of mycelia by thin layer chromatography (TLC) and HPLC analysis that could be accounted for the aberrant cell wall formation and the elevated osmotic sensitivity.

In case of Δ*AnhmgB-B* strain we observed abnormal morphology of the mycelia, drastic decreasement of viability of the asexual spores (52% in wt and 0.35% in mutant at 37°C) and increased sensitivity of the mycelia to oxidative stress. The spores of wild-type and mutant strains were subjected to metabolite analysis by GC-MS analysis that revealed a singinficantly lower level of xylitol and trehalose content in the deleted spores, which could explain the decresed viability. To find out the reason of the oxidative stress sensitivity of mutants, SOD (superoxide-dismutase) content of deleted and wild-type strains were compared, and significant differences in relative ratio of SOD isoenzymes were detected.

The mycelia of double-deleted mutant showed nearly complete inhibition of growth in thermo-stress condition (at 42°C). To find out the cause of the thermosensitivity, a comparative transcriptome sequencing was carried out by „Next Generation Sequencing" (NGS). The analysis process of transcriptom data are in progress. The preliminary results has already revealed that the mutant strain cannot maintain the expression activity of that of wt either at 37 or 42°C. It seems that the transcriptional discrepancies affect the whole chromatin in the mutant. For example, wt strain increases the transcription of 293 genes and decreases the transcription of 540 genes when temperature is shifted from 37 to 42°C. When these data are compared to that of the mutant we observed that out of the normally upregulated 293 genes in wt 52 genes were downregulated and 82 genes were overexpressed. Similarly, transcription activity changed in the downregulated population of 540 genes, where 94 genes were upregulated instead of downregulation and 123 genes were significantly downregulated than that was observed in the wt. As a preliminary result of the NGS analysis the metabolic pathways leading to secondary metabolite production was assumed to be disturbed in the mutant. To prove our finding experimentally, the total metabolite composition of wt and mutant strain was monitored by TLC and we found that certain intermediate metabolic compounds are extremely accumulated in the mutant strain. We expect that several further experiments will be carried out on the basis of the results of the transcriptome analysis. In the future, we would like to purify the two proteins to carry out „pull down" assays and DNA-binding experiments. The identification of the interacting proteins and DNA sequences would give a deeper insight into the function of the proteins at the molecular level.

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Changes in plant antioxidants and photosynthesis in response to abiotic stresses

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As a consequence of their sedentary habit, plants are exposed to changing environmental conditions which may cause stress. A common effect of these abiotic stress factors is the rapid generation of reactive oxygen species (ROS) which are also found at very low concentrations in unstressed plants. Plants have evolved different enzymatic (SOD, POD, etc.) and non-enzymatic (ascorbic acid, phenols, carotenoids, etc.) antioxidants to reduce the amount of these potentially harmful agents.

The elevated amount of ROS in plants is a signal of the antioxidant system being overwhelmed and is thus an important indicator of severe stress. Under these conditions, ROS are detectable directly, by a variety of modern biophysical methods. These, however, are not sensitive enough for detecting the ROS assumed to accompany moderate, acclimatory stress. Under these conditions, measuring changes in activity and quantity of the antioxidants is a widely used stress indicator in plant physiology. The aim of our work was to connect antioxidant

content, specific ROS scavenging capacities and acclimation potential of leaves under various moderate stress conditions.

Methods already exist for measuring total antioxidant capacities and for detecting enzymes specific to a certain ROS (like peroxidases or superoxide dismutases), however some of the ROS are not aimed by specific enzymes and are scavenged by the common effect of different compounds. Therefore we developed new methods for measuring antioxidant capacities of plant samples specific to the hydroxyl radical (Majer et al. 2010b) and the singlet oxygen, (Hideg and Majer 2010). By correlating the ROS specific and total antioxidant parameters we showed the importance of studying the former besides measuring the widely used latter parameters as well (Majer et al. 2010b). In addition, we developed a new method which serves us with an initial screening of photosynthesis in leaves based on digital images (Majer et al. 2010a).

Using the above techniques and chlorophyll-fluorescence-based assessment of leaf photochemistry, we studied contributions of preventive and antioxidant processes to high-light tolerance in linden tree (*Tilia platyphyllos* L.) leaves collected from sun-exposed and shaded parts of the same tree. According to our results, linden sun leaves had 2-times stronger singlet oxygen neutralizing capacities than shade leaves and were able to avoid non-regulated loss of energy under high PAR (Hideg and Majer, 2010). In sun linden leaves significantly higher amounts of flavonoid glycosides were found and contributions of various phenolics to specific ROS capacities are currently investigated.

In an other series of experiments we studied the acclimation potential to UV-B radiation in younger and older grapevine leaves (*Vitis vinifera* L. cv. Chardonnay) and found that younger leaves were able to mobilize screening pigments and antioxidants better and therefore suffered less damage from UV-B as compared to older ones (Majer and Hideg 2011).

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The role of oxidative stress in antibiotic resistance evolution

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The emergence of bacteria that are resistant to multiple antibiotics represents an increasingly significant threat to public health today. There are a number of mechanisms whereby bacteria can develop antibiotic resistance including the physical exchange of genetic material with another organism, the activation of latent mobile genetic elements (transposons or cryptic genes) and the mutagenesis of its own DNA. Chromosomal mutagenesis may arise directly from antibiotic-induced oxidative stress, or indirectly, as a consequence of the interaction between antibiotic and its target molecule or from the activity of bacterium's error-prone DNA polymerases during the repair of DNA lesions.

There are at least three major mutational mechanisms which facilitate gradual evolution of bacterial antibiotics resistance. These mechanisms include mutators (genotypes with increased, constitutive mutation rates), SOS response (a global response that minimizes the lethal and mutagenic consequences of the exposure of cell to DNA damaging agents) and the direct mutagenic effect of reactive oxygen species (ROS). We investigated how far these three above mentioned mechanisms are linked to each other, and what their relative contribution to antibiotics resistance evolution is.

It is known from literature that bactericidal (but not bacteriostatic) antibiotics share a common lethal pathway that involves the generation/accumulation of ROS. In our study we focused on the role of ROS in antibiotic resistance evolution. The effect of ROS has a double edged sword feature. On one hand ROS production contributes significantly to the killing effect of bactericidal antibiotics. On other hand, by directly damaging DNA, ROS accumulation increases the mutation supply of the bacterial cell which promotes the appearance of antibiotic resistant strains.

We employed a series of 10-15 days-long laboratory evolutionary experiments with *E. coli BW25113.* The used strains were initially sensitive to antibiotics and differ only in their respective constitutive genomic mutation rates (wild type *BW25113* versus ΔmutS) and/or activities of the SOS response (wild type versus LexA3 expressing strain or ΔmutSLexA3). 96 independent lineages of each bacterial strain were allowed to evolve in microtiter plates to successively higher antibiotic concentrations by transferring daily 1% of each culture. We employed three antibiotics: ciprofloxacin, ampicillin, tobramycin, representing three major classes of bactericidal antibiotics (quinolones, β-lactams and aminoglycosides respectively) known to stimulate the production of ROS. As a negative control, we also tested the bacteriostatic trimethoprim, a folic acid biosynthesis inhibitor. In order to test the contribution of ROS formation on evolvability we added thiourea to the medium. Thiourea is a hydroxyl radical scavenger which mitigates the damage caused by ROS formation upon antibiotic treatment.

We found that thiourea substantially promotes extinction of evolving bacterial populations for all three cases of bactericid antibiotics treatments both in the case of mutators and non-mutators. Our results indicate that scavenging of reactive oxygen species by thiourea substantially reduces mutational input, and hence the corresponding populations had lower opportunity to evolve resistance against the used antibiotic. Thiourea treatment had no significant impact on extinction rates of the populations adapted to the bacteriostatic drug trimethoprim. We found that the impact of ROS formation on resistance evolution is at least partially independent of the SOS response. Our work suggests that enhancement of ROS formation may be useful for improving antibiotics lethality in the short term, but it also accelerates drug resistance evolution.

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Investigation of a novel stress-induced operon in *Synechocystis* PCC 6803

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Cyanobacteria are photosynthetic prokaryotes that are widely used model organisms of photosynthetic research according to their close relationship to chloroplasts. These prokaryotes possess a variety of adaptation mechanism by which they are able to inhabit different extreme biotops. Since they are autotrophic organisms they are the primary producers of these niches. Among others, arsenic compounds and hydrogen sulfide are widely distributed in nature since the origin of life. Cyanobacteria have evolved mechanisms to cope with the toxicity of these substances. In order to maintain their homeostasis under fluctuating environmental changes cyanobacteria evolved environmentally inducible detoxification systems.

We investigated the expression pattern of different genes upon heavy metal treatment in *Synechocystis* sp. PCC 6803 and discovered a novel gene cluster that responds to As^{3+}. This operon is located on the pSYSM plasmid and contains four genes, termed *artRSCT*. *ArtR* and *artS* are transcribed in the opposite direction of that of *artC* and *artT*, with a common promoter region between these two tandem gene pairs.

The gene product of *artR* shows high homology with DNA-binding proteins of the ArsR family. We have constructed a deletion mutant in order to inactivate the *artR* gene. In this mutant we observed high constitutive expression of the *artS/C/T* genes using quantitative RT-PCR technique. This fact supported our hypothesis that it functions as a common repressor of the genes. We have cloned and expressed this protein in *E. coli* in order to obtain further proof of its function. Using this purified protein we performed an electrophoretic mobility shift assay, which confirmed the specifity of ArtR towards the promoter sequence. Taken all these results into consideration we have proven that the protein product of *artR* is a regulator of the *artRSCT* operon, which binds to the common promoter and dissociates in the presence of As^{3+}.

ArtT shows high homology with membrane-bound heavy metal transporter proteins and because it is induced by As^{3+}, we assumed that it may be part of an arsenic resistance system. In order to test this hypothesis we have constructed an *artT* insertional mutant. The results were contradictory to our hypothesis because the mutant was more resistant to elevated amounts of As^{3+} then the wild type. Thus the function of *artT* may be different from that of the arsenic resistance genes.

The protein product of *artS* gene is homologous to sulfide-quinone oxido-reductases (SQR) and shows a high expression not only to As^{3+} but to Na_2S as well. Moreover *Synechocystis* PCC 6803 was more resistant to high amounts of Na_2S then other cyanobacterial species that lacks the SQR enzyme.

H_2S and As^{3+} can serve as alternative electron donors in many ancient anaerobic photosynthetic systems. The procaryotes capable of this type of electron transport use a single photosystem (PSI) and harbor a SQR enzymes, which oxidize sulfide to sulfur. Other strains can use arsenite oxidase enzyme to grow phototrophically in As^{3+} containing anaerobic environment. The facts that the *artRSCT* genes are induced by As^{3+} and Na_2S and the strain is more tolerant to Na_2S, than those which lack such genes suggest that these genes, especially *artS* may be involved in sulfide tolerance or sulfide metabolism of the cyanobacterium.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Role of the glutamine synthetase isoenzymes in abiotic stresses

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In plants, glutamine synthetase (GS, EC 6.3.1.2) is the key enzyme of primary nitrogen assimilation, as well as ammonia reassimilation and detoxification. GS catalyses an ATP-dependent conversion of glutamate to glutamine using ammonium derived from fertilizer, nitrate reduction, photorespiration in C3 plants, and numerous other sources including the catabolic release of ammonium during senescence. Plants have two different isoenzymes located in the chloroplast (GS2) and the cytosol (GS1). In young leaves, GS2 plays a major role in fixing ammonium into amino acids. In old, senescing leaves it is proposed that glutamine is synthesized by GS1 isoforms.

Soil acidification and drought stress are the most important abiotic factors in agriculture. A decrease in soil pH may release water soluble, toxic aluminium species from clay minerals. Al interferes with a wide range of physical and cellular processes. Plant GS requires two magnesium ions per subunit for activity, which makes GS a potential target of metal stress. The objective of the first investigation was to prove that Al from an organic metal complex is able to activate GS, and Al becomes bound to the polypeptide structure of the GS molecule. Aluminium(III)–nitrilotriacetic acid complex (Al(III)NTA) activated the GS prepared from wheat (*Triticum aestivum* L.) leaves, as Al^{3+} did in vivo, but could not functionally substitute magnesium ions, which were also necessary for the activity in the in vitro GS assay. GS2 was isolated by non-denaturing polyacrylamide gel electrophoresis, and the Al and Mg content of the enzyme was determined by inductively coupled plasma atomic emission (ICP) spectroscopy. The GS octamer remained intact and contained Mg^{2+} bound to its specific sites after the electrophoretic separation. Al was detected in the Al(III)NTA-treated sample bound to the structure of the enzyme protein, potentially occupying one of the specific metal-binding sites of the subunits. Our results indicate that the activator effect of the Al(III)NTA complex is due to the specific binding of aluminium to the polypeptide chain of GS2; however, the presence of magnesium at least on one of the metal-binding sites is essential for the active state of the enzyme.

Drought stress may have a considerable impact on the ecosystem and agriculture. Drought stress induces early leaf senescence. During this process, chloroplasts are degraded and photosynthesis drastically drops. The objective of this investigation was to look into the regulation of nitrogen and carbon metabolism during water deficit stress. GS isoenzymes are good markers of the plastid status (GS2) and the nitrogen metabolism (GS1). Tolerant and sensitive wheat genotypes were tested, which are widely used in agriculture. The amount of Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) and GS isofroms in leaves were measured during the grain filling period, as indicative traits that ultimately determine the onset and stage of senescence. The symptoms of senescence appear first on the oldest and finally on the youngest leaves. The sequentiality of senescence was disrupted in the sensitive varieties during drought stress. In the flag leaves an untimely senescence appeared, earlier than in the older leaf levels. Total protein and Rubisco content decreased and the GS2 isoenzyme disappeared. These physiological parameters did not change in the tolerant varieties under drought as compared to the control, well watered plants, or only the gradient of senescence became steeper, indicating the acceleration of this process.

Our results revealed the indicator role of GS in different abiotic stresses, which can be applied for characterization (classification) of wheat cultivars in terms of abiotic stress tolerance.

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Focusing on the fuction of endogenous galectin-1 produced by activated T-cells

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The immune system is a tightly regulated network that is able to maintain a balance of immune homeostasis under physiological conditions. Normally, when challenged with foreign antigen, specific response is initiated which aims restoring homeostasis. In this regard the final step during immune response is the elimination of the antigen activated specific lymphocytes by apoptosis. Death of the already functionless cells may occur via different mechanisms including galectin-1 (Gal-1), a β-galactoside binding mammalian lectin, which has been implicated in T-cell apoptosis. Recent studies from our and other laboratories showed that Gal-1 induces apoptosis of activated peripherial inflammatory Th1 cells, hence participating in the down-regulation of the T-cell response. This cytotoxic effect of Gal-1 is exerted by secreted, extracellular protein acting on Gal-1 binding structures on activated T-cells. On the other hand Gal-1 is expressed in activated but not resting T-cells. However the cellular localization and function of the *de novo* expressed Gal-1 in activated T-cells has not been revealed yet. Our aim was to determine the role of T-cell derived Gal-1 in the regulation of T-cell viability.

First we established Gal-1 transgenic Jurkat T-cell line, JGal as a model of activated T-cells. Using JGal, we demonstrated that the transgene Gal-1 remained intracellularly and these cells became more sensitive to apoptosis induced by extracellular exogenous Gal-1 delivered either as soluble or HeLa cell-derived protein. Moreover other cytotoxic reagents, such as hydrogen-peroxide and staurosporine induced lower apoptotic response compared to that of the non-transgenic (Gal-$1^{-/-}$) Jurkat cells.

Then we analyzed Gal-1 expression in activated peripheral T lymphocytes. In physiological T-cells Gal-1 was expressed upon activation, remained intracellularly and as a consequence of Gal-1 expression, activated T-cells responded with apoptosis to extracellular Gal-1. T-cells were also isolated from patients suffering from an autoimmune disease, systemic lupus erythematosus (SLE). We found that SLE T-cells failed to express Gal-1 upon activation and accordingly, these cells were more resistant to apoptosis induced by exogenous Gal-1.

Our results show that Gal-1 transgenic T-cell line, JGal resembling activated T-cells and physiological but not SLE activated T-cells express but do not secrete galectin-1. T-cells producing intracellular Gal-1 become more sensitive to apoptosis triggered by extracellular Gal-1. These data strongly indicate that intracellular Gal-1 in activated T-cells contribute to the fine regulation of the termination of immune response and additionally, the failure of Gal-1 expression in autoimmune T-cells may regulate the disease onset..

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Obesity research regarding lifestyle and socio-economic background among people of different age groups

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Obesity defined as excessive or abnormal fat accumulation has reached epidemic levels in developed and developing countries too. The prevalence of obesity has risen considerably over the past three decades, and this trend continues nowadays. According to the Word Health Organization approximately 1.5 billion adults were overweight globally in 2008, more than 200 million men and nearly 300 million women of which were obese. Furthermore, this problem does not only affect adults, but also children and adolescents (WHO 2010). Increased risk of a number of life threatening diseases is linked to obesity, such as cardiovascular diseases, diabetes mellitus, musculoskeletal disorders, different forms of cancers. In addition to health problems, obesity is also a serious financial burden both for the society and for the obese individuals (Bray 2004, Thompson et al. 1999).

The fundamental cause of obesity and overweight is an energy imbalance between energy intake and energy expenditure. Although the genetic background of obesity development is indisputable, and research advances have highlighted the importance of molecular genetic factors in determining individual susceptibility to obesity, it can not explain the worldwide obesity epidemic. There is supporting evidence that different environmental factors, lifestyle preferences (global shift in diet towards overconsumption, and a trend towards decreased physical activity), cultural and socio-economic environment seem to play major roles in the rising prevalence of obesity (Stein and Colditz 2004). The purpose of our study was to examine the relationship between the nutritional status and certain socio-economic and lifestyle factors among people of different age groups.

596 high school students (aged 14-18) and 1205 university students (aged 18-25) participated in the examination. To estimate overweight, obesity and fat distribution, we determined body mass index (BMI), waist circumference and wais-to-hip ration (WHR). We used questionnaires to gather the required information about the participants' lifestyle and socio-economic background.

Some of our key findings were: the prevalence of obesity was 14,3% among the high school students (15,36% of boys and 12,41% of girls). 22% of university students were overweight (males: 33,1%; females: 15,5%), and 5,2% of them were obese (males: 7,7%; females: 3,8%). The result showed that parental education level was related to the students overweight (lower level associated with higher BMI). The prevalence of overweight and obesity decreased by number of daily meals, and the normal weight students showed more frequently eating sweets than the overweight group. The majority of examined high school students had weak physical abilities, and the Hungarofit scores were negatively associated with BMI. The prevalence of overweight and obesity in adolescents living in a large city (Szeged) was significant fewer compared to teenagers living in small town area (Jánoshalma), and the former spent significantly less time in front of the TV and computer in their free time.

Additionally, to examine the benefits of regular exercise, 172 volunteers were recruited and enrolled in a 4-month physical activity intervention programme. The decrease in BMI and percent body fat loss was significant.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Salt stress and salicylic acid induced programmed cell death in tomato

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Programmed cell death (PCD) is an integral part of the plant development and in the response to changing environments. PCD is induced by various abiotic stressors such as high salinity. A special type of PCD in plants is the hypersensitive response during biotic stress which is mediated by salicylic acid (SA). Salt stress results in the disturbance in ion homeostasis, water status and redox equilibrium of plant cells. SA can also cause changes in the water status of plants and can result in oxidative stress but it is not known how SA affects the ion homeostasis of cells. The aims of our work were to study the effects of different concentrations of NaCl and SA on the cell death initiation in tomato in order to highlight the common features or differences in the NaCl- and SA-induced PCD.

Supraoptimal concentrations of NaCl and SA increased the amount of reactive oxygen species (ROS) and H_2O_2 content of leaf tissues. The inhibition of photosynthetic electron transport can significantly contribute to the generation of ROS in chloroplasts. The photosynthetic performance can be controlled by the limitation of CO_2 diffusion through stomatal pores. The addition of NaCl at 100-250 mM and SA at 10^{-7}-10^{-2} M to the hydroponic culture of tomato plants for 6 hours resulted in stomatal closure on intact leaves. In parallel with stomatal closure 100-250 mM NaCl and 10^{-3}-10^{-2} M SA decreased the maximal CO_2 fixation rate (A_{max}), and the initial slopes of the CO_2 (A/C_i) and light response (A/PPFD) curves and relative electron transport rate (Rel. ETR) in intact leaves. Those concentrations of SA which decreased the photosynthetic performance in intact leaves led later to PCD.

Our work aims to investigate whether SA has direct control over stomatal movement by increasing the levels of ROS and nitric oxide (NO) in guard cells or by changing the photosynthetic activity of stomata. In contrast to leaves, stomata on the abaxial epidermal peels closed in buffers containing 10^{-7} and 10^{-3} M SA but remained open at 10^{-4} M. At those concentrations which induced stomatal closure ROS and NO levels raised which could be prevented by ascorbic acid, catalase and diphenyleneiodonium or cPTIO, the scavengers of ROS and NO, respectively. In contrast, the lack of a permanent ROS accumulation and the decrease in NO production in guard cells promoted stomatal opening at 10^{-4} M SA. SA at higher concentrations inhibited the Rel. ETR in guard cell chloroplasts suggesting a decrease in photosynthetic performance of guard cells.

The specific genes involved in cell death program were induced by NaCl and SA and their expression levels were analysed by RT-PCR. The expression of both the inhibitors (e.g. BAX-Inhibitor) and effectors (e.g. cysteine proteinases) of PCD were significantly enhanced at lethal concentrations of NaCl and SA. These results suggest that the PCD in these tissues can be triggered in spite of the high expression level of PCD-inhibiting genes.

Signal transduction pathways induced by salt stress and SA have been compared at cell level in tomato cell suspension culture. 250 mM NaCl and 10^{-3} M SA caused the death of tomato suspension cells within 6 hours which was accompanied by DNA fragmentation. Our results show that supraoptimal concentration of NaCl induced cell death by generating ionic- and oxidative stress and inducing ethylene production but SA induced cell death by generating oxidative stress.

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The Type II restriction endonuclease MvaI has dual specificity

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Type II restriction endonucleases (REases) are sequence-specific endonucleases that recognize short DNA sequences and cut the DNA at defined positions within or close to the recognition sequence. In the producer cell the host DNA is protected by specific methylation of the recognition sequence. Methylation is established by DNA methyltransferases, which methylate a cytosine or adenine to produce C5-methylcytosine, N4-methylcytosine or N6-methyladenine. This huge group of enzymes shows great diversity. Members are classified into subgroups according to the symmetry of their recognition sequence, the position of the cut site relative to the recognition sequence, the number of target sites the enzyme interacts with, etc.

From the perspective of our study, two subgroups of Type II REases are especially interesting. Enzymes in the Type IIM subgroup (methyl-directed REases) break the general rule of protection by DNA methylation; unlike most restriction endonucleases, they require methylated substrate site for activity. The other interesting subtype are nicking REases, which cut only one strand of the substrate DNA. Such enzymes include natural nicking REases, e.g. N.BstNBI, isolated subunits of heterodimeric REases (Nb.BsrDI, Nb.BtsI, and mutant REases engineered to cut only one strand of the substrate (Nt.AlwI).

The MvaI REase recognizes the sequence CC↓WGG (W stands for A or T) and cuts both strands as indicated, generating one nucleotide 5'-overhangs. The cognate DNA-methyltransferase M.MvaI modifies the internal cytosines to produce N4-methylcytosine: (C^{m4}CAGG/ C^{m4}CTGG). MvaI was shown to recognize its pseudosymmetric target site as a monomer. An interesting feature of the enzyme is its toler-

ance to a wide range of modifications within the recognition sequence. MvaI shares ~20% sequence identity and structural similarity with BcnI, a REase recognizing the related pseudopalindromic sequence CC/SGG (S stands for G or C).

Here, we show that MvaI has two specificities: in addition to cutting its well-known recognition site (CC↓AGG/CC↑TGG), it can nick the related C<u>C</u>↓GGG/CC<u>C</u>GG sequence (BcnI site) if the underlined cytosines are C5-methylated (C<u>C</u>↓GGG/CC<u>C</u>GG). The single-strand scission occurs in the G-strand as indicated. At sequences, where two oppositely oriented methylated BcnI sites partially overlap (SmaI sites), double-nicking leads to double-strand cleavage (CC^{m5}C↓GGG/CC^{m5}C↑GGG), generating fragments with blunt ends. The double-strand cleavage rate at these sites is ~five to tenfold lower than at the canonical target sites.

MvaI is the first restriction enzyme, for which activity on an unmethylated as well as on a methylated substrate site has been shown. The new, methylation-dependent activity represents nicking and double-stranded cleavage specificities (C^{m5}C↓GGG/CC^{m5}CGG and CC^{m5}C↓GGG/CC^{m5}C↑GGG, respectively) not known before.

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Automated refinement of a genome-scale metabolic model of yeast based on high-throughput genetic interaction data

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Genome-scale stoichiometric models are *in silico* representations of the metabolism of living organisms. Genetic and conditional changes can be introduced to these models and the effect of these modifications can be investigated. Certain phenotypes, like single gene deletion, can be predicted with high accuracy; however, it has remained untested whether the metabolic models can also successfully capture genetic interactions (*i.e.* non-independence between mutation effects). Genetic interactions have two major forms: positive (or alleviating) and negative (or aggravating) epistasis. Positive interactions occur when deletion of two genes simultaneously has a higher fitness than would be expected based on the fitness effect of the single mutations (e.g. if two genes are in the same linear pathway the second mutation has no additional fitness effect). Similarly, genes show negative interaction when the double mutant has significantly lower fitness than the combined effect of the single deletions (e.g. synthetic lethality, when the single mutants are viable but the joint deletion of the genes is lethal).

To determine the in silico model's accuracy to predict genetic interactions we systematically compared computational predictions with a unique genetic interaction dataset generated, as part of a collaboration, by the Boone lab[1] and which comprises ~185,000 metabolic gene pairs. In the *in silico* analyses we used a *Saccharomyces cerevisiae* metabolic reconstruction containing 904 genes (iMM904)[2]. The metabolites and reactions are represented by their stoichiometric coefficients and the model contains information on reaction reversibility, however, it does not incorporate kinetic details. We applied the widely used flux balance analysis (FBA) modelling tool to compute mutant fitness (*i.e.* biomass production efficiency).

We found that several properties of the *in vivo* genetic network were successfully captured by the model (e. g. single mutants with severe fitness defects tend to show many genetic interactions); however, it recovered only a minority of experimentally observed interactions.

Because our knowledge of metabolism is certainly imperfect, we sought to improve the prediction performance of the metabolic model and developed an optimization-based algorithm to automatically refine the network based on empirical genetic interaction data. Our method suggested several modifications and we experimentally verified some of them. For example, the essentiality of the kynurenine pathway genes (*BNA1*, *BNA2*, *BNA4*, and *BNA5*) in the absence of nicotinic acid was undetected by the original model due to the erroneous presence of a NAD biosynthesis route. Finally, based on our algorithm and on literature data, we substantially revised the NAD biosynthesis pathway of the genome-scale metabolic reconstruction of yeast.

Our work has recently been accepted for publication in *Nature Genetics*.

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Volume 55(1):197-212, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Characterization of molting defective gene in the ecdysone production of Drosophila larvae

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Steroid hormone ecdysone (E) mediates a wide variety of developmental events in insects. Therefore, understanding the function and the regulation of ecdysone signalling pathway is essential for insect biology. During Drosophila larval life the source of ecdysone is the endocrine organ, the ring gland (RG). Ecdysone is synthesized from cholesterol via a series of hydroxylation steps catalyzed by cytochrome P450 enzymes.

Known genes encoding Ecdysone synthesis enzymes (spook, phantom, disembodied, shadow and shade) are members of the „Halloween" class of genes. Mutants of this class share a characteristic phenotype: thin, unstructured embryonic cuticle with dorsal and anterior holes therefore caused embryonic lethality. Although several studies reported details of ecdysone synthesis, little is known about the regulation of the process. Here we report how the *molting defective* gene (*mld*) is involved in the regulation of ecdysone production.

Mld encodes a nuclear zinc finger protein that is required for ecdysone production. Since *mld* does not regulate the expression of known ecdysone synthesis genes, it might control the expression of steroid biosynthesis. Mutations of *mld* cause an arrest of development in the first larval instar. This defect can be rescued by providing ecdysone.

Mld homozygous mutant larvae have two main phenotypes. On the one hand it has an enlarged ring gland and on the other hand it is lack of ecdysone.

We show that the hypertrophy of RG is due the increased polytheny of PG cells. Using somatic mosaics we also showed that RG hypertrophy is not autonomous. The PG hypertrophy is due to the low ecdysone level in the mutant, and suppressed if treated with ecdysone. This effect is inhibited if ecdysone receptor gene (EcR) is silenced in PG cells by RNAi. Silencing EcR isoforms in RG specific manner in wild type also has an organism specific effect, resulting enlarged RG and smaller adult body size.

We conclude from these results that *mld* gene is a larval specific regulator of ecdysone production. We found, the phenocritical period of *mld* mutant phenotype is in the second part of the first larval stage, the time when ecdysone production is needed, also supports the larva specific role the gene. Furthermore the results of epistasis experiments with *mld*, *sad* (E synthesis gene), and mld, *kkv* (ecdysone dependent cuticle biosynthetic gene) show that *mld* does not play role in embryonic development. Studies with germ line mosaics of amorphous *mld* alleles indicate the lack of maternal effect of mld gene.

We found that the classic ecdysone deficient mutation DTS3 (dominant temperature sensitive-3) is an *mld* allele. In order to better understand the DTS phenotype we determined the mutation sites of mld^{DTS3} by sequencing the mutant allele. Two point mutations were found in exon-three, one resulting a premature stop codon, and an other resulting P to S amino acid change.

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Influence of DNA damage and repair, on the ability of cyanobacterial cells to repair UV-B radiation-induced damage to the Photosystem II complex

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Two of the most significant primary effects of UV-B irradiation in cells of photosynthetic organisms are the damage to DNA and the impairment of active protein complexes, of which the most pronounced one is the inactivation of Photosystem II mainly due to damaging the D1 protein. We have investigated the correlation of Photosystem II protein damage and its repair with the concomitant DNA damage and its repair. As model organisms the cyanobacterium *Synechocystis* PCC6803 wild type (WT), as well as its photolyase lacking mutant (ΔphrA) were used for this purpose. We found that during exposure to UV-B radiation the ΔphrA cells accumulated a significant number of DNA damages concomitant with a radical decrease in Photosystem II activity, and D1 protein levels. After terminating the UV-B illumination the ΔphrA cells showed no repair of damaged DNA, and only a limited capacity to repair the damaged Photosystem II centers. The WT cells, however, didn't suffer significant damages to their DNA. In these cells PSII activity as well as repair capacity, including effective turnover of the D1 protein pool, was maintained under the same UV-B irradiation conditions. These data show that the repair capacity of Photosystem II is directly influenced by the ability of cells to repair UV-B damaged DNA.

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