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

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

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

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

Supervisor: Jolán Csiszár
E-mail: brunner.szilu@gmail.com

Protecting roles of 27 kDa heat shock protein Hsp27

Balázs Csibrány

Laboratory of Animal Genetics and Molecular Neurobiology, Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

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

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

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

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

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

Supervisor: Miklós Sántha
E-mail: santha.miklos@brc.mta.hu

Regulation of protective proline synthesis during reactive carbonyl stress

Aleksza Dávid

Laboratory of Molecular Regulators of Plant Growth, Department of Plant Biology, Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary

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

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

Supervisor: Gábor V. Horváth
Email: aldoaat@gmail.com

Sulfide oxidizing enzymes in a purple sulfur photosynthetic bacterium

Ágnes Duzs

Department of Biotechnology, University of Szeged, Szeged, Hungary

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