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)

and a smaller, heme c binding cytochrome c subunit (FccA). Sulfide quinone oxidoreductases are monomeric membrane-bound flavoproteins which present in all domains of life. Sqr can transfer electrons from sulfide directly into the membrane quinone pool while Fcc reduces periplasmic c-type cytochrome proteins.

Thiocapsa roseopersicina is a photosynthetic purple sulfur bacterium. Three genes encoding sulfide oxidizing disulfide oxidoreductases were identified in the genome sequence: *fcc*, *sqr* and *sqn*. The Sqr and Sqn belong to group IV and group VI of the Sqr-type proteins, respectively. A detailed comparative biochemical, structural and functional analysis of these proteins is in the focus of this study.

The FccAB complex, the FccB, the Sqr and the Sqn proteins fused to Strep II affinity tag were expressed in *T. roseopersicina* strains. The recombinant flavocytochrome c variants and the Sqn enzyme could be purified to homogenity by affinity chromatography. In the absorption spectra of the oxidized and reduced forms of FccB, FccAB and Sqn, characteristic peaks of redox active flavin prosthetic group were identified. The flavin moiety apparently bound covalently to the proteins. The flavocytochrome c had also a redox active heme cofactor non-covalently bound to the FccA subunit. The Fcc variants were subjected to ultrafast fluorescence kinetic measurements in order to determine the interaction between the FAD cofactor and the protein. The affinity purified recombinant FccAB could oxidize sulfide and was able to reduce bovine heart cytochrome c at low sulfide concentrations. The temperature and pH dependences of the activity of the recombinant Fcc complex were determined: the optimal temperature was 45 °C while the optimal pH was 8.0. The FccAB was a moderately thermostable enzyme which had remarkable activity up to 60 °C. The recombinant Sqn and Sqr catalyzed the sulfur-dependent quinone reduction. The temperature and pH optima of quinone reductase activity of the Sqn were the same as determined for FccAB. Kinetic analysis of the Sqn activity at various pH revealed a lag phase preceding the reaction at high pH. This might mean that the enzyme needed activation for being able to reduce quinones at alkaline conditions. Additionally, the macromolecule structure of the Sqn was analyzed to explore the connections between the quaternary structure and the catalytic properties of the protein. Enzyme kinetic parameters of the Sqn disclosed that the enzyme affinity for sulfide was low as compared to other well-known sulfide quinone oxidoreductases. Consequently, Sqn might play role in the sulfide oxidation at high sulfide concentration. In contrast, the FccAB could have important function at low sulfide concentration in the sulfur metabolism in *T. roseopersicina*. The structural and functional analyses of the wild and mutant flavocytochrome c might lead to better understanding of the structure/function relationships of the disulfide oxidoreductase protein family. On the other hand, the biochemical and biophysical characterization of the Sqn should disclose specific properties of the group VI. of the Sqr-type proteins.

Supervisor: Gábor Rákhely, András Tóth
E-mail: duzs.agnes@gmail.com

Heavy metal induced nitro-oxidative stress in *Brassica* species

Gábor Feigl

NO-signalling group, Department of Plant Biology, University of Szeged, Szeged, Hungary

Copper (Cu) and zinc (Zn) are essential micronutrients, which can be present in soils naturally or can be accumulated in the environment due to anthropogenic activities. Cu is a redox-active element, directly inducing the formation of reactive oxygen species (ROS) leading to oxidative stress. Zn, on the other hand, is a non-redox-active element, causing oxidative stress indirectly by the modulation of antioxidant capacity. Moreover, in excess, both metal trigger changes in the metabolism of reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite ($ONOO^-$) leading to nitrosative stress. The oxidative and nitrosative signalling interact with each other resulting nitro-oxidative stress during which the cellular functions damage by lipid peroxidation and nitration, protein carbonylation, tyrosine nitration and S-nitrosylation.

The primary goal of my study was to determine the degree of nitro-oxidative stress in two metal tolerant *Brassica* species exposed to Cu or Zn. Furthermore, I wanted to draw conclusions about the Cu- and Zn tolerance and phytoremediation usability of the species.

Nine-days-old hydroponically grown *Brassica juncea* and *Brassica napus* were treated with 0 (control), 10, 25 and 50 µM $CuSO_4$ or 0 (control), 50, 150 and 300 µM $ZnSO_4$ in nutrient solution for 7 or 14 days. Changes in microelement contents, formation of different ROS and RNS, cell viability, lipid peroxidation, cell wall alterations and enzymatic- and non-enzymatic antioxidants were examined in the root system.

Most of the Cu and Zn taken up by the plants were retained in the roots; however, the increment of Cu and Zn content within the *Brassica* shoots indicated an efficient translocation. Both metals in excess markedly modified the microelement homeostasis of *Brassica* plants. Both Cu and Zn treatment caused significant morphological alterations in the root system of *Brassica* species, e.g Cu and Zn were able to increase the lateral root number, especially in *B. juncea*, which may be part of a morphological adaptation process. A Cu concentration-dependent decrease of cell viability was also found after both 7 and 14 days of treatment; however in short term *B. juncea* root meristem did not show Zn-induced viability loss. Also, cell wall alterations were notable, since intensified lignification and callose formation were detected in the root system of Cu-stressed plants; however excess Zn caused only increased callose deposition.

Exposure to Cu induced nitric oxide generation in the root tips and this event proved to be dependent on the duration of the exposure and on the plant species. In short-and long-term treatments, *B. juncea* showed more significant activation of superoxide dismutase (SOD), inhibition of ascorbate peroxidase (APX) and oxidation of ascorbate (AsA) than *B. napus*. Moreover, hydrogen peroxide (H_2O_2)-dependent lignification was also observed in the Cu-exposed plants. In longer term, significant AsA accumulation and callose deposition were observed,