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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

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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