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Functional characterization of candidate genes in barley: transgenic plants and grown cultivars

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Barley (*Hordeum vulgare L.*) is one of the major and most distributed crops in the world. Currently it is becoming a novel cereal model plant representing a number of small-grain cereal species. While the barley genome is similar to that of other cereals, it is amenable to explorations of molecular genetics through its true diploidy.

The first Agrobacterium-mediated barley transformation reports was published in 1997 by Tingay and co-workers, using the variety called Golden Promise. The method that we established here was developed for this model cultivar and it's based upon protocols by Trifonova et al. (2001) and Kumlehn (IPK, Gatersleben, unpublished).

The selected genes we used for the plant transformations can be divided into the following three subgroups:

1. The regulators of the cell divison cycle: *MsCDKB2;1* (a *Medicago sativa* cyclin dependent kinase which plays a central role in regulation of the cell cycle, in particular in the G2/M phase transition and in mitosis). In previous studies it revealed that the overproduction of the *MsCDKB2;1* resulted in significant changes in agronomically important parameters in transgenic rice (Lendvai et al., unpublished). Other genes of this group, like OsPP2A B" regulatory subunit, OsRBRI2, OsRBRI5 are previously identified interactors of rice retinoblastoma-related protein, OsRBR1. Since cell cycle regulatory functions of the retinoblastoma proteins are primarily modulated by changing their phosphorylation status, *in planta* studies of the OsRBR1 interaction partner, the OsPP2A protien phosphatase B" regulatory subunit is particularly important from this viewpoint.

2. The 'oxidative stress-defense genes'. First transformation from this group of genes were made by the alfalfa aldo-keto reductase, *MsALR*. This enzyme plays important role in detoxification of the reactive aldehydes issued during oxidative stress, and helps the recovery of the plants (Oberschall et al. 2000). In order to accumulate protective enzymes in different subcellular compartments we constructed a vector for chloroplast targeting of protective enzymes using the transit peptide encoding region of the barley Rubisco LSU gene.

3. The genes involved in grain size determination (*GW2*, *GIF1*). Loss of *GW2* function increased grain width, weight and yield (Song et al. 2007) Antisense approach results increased grain size, even with constitutive expression of gene fragment in transgenic rice. We have identified and cloned the homologous gene from barley, a specific fragment of it was used for the generation of *HvGW2* antisense plants. *GIF1* (*GRAIN INCOMPLETE FILLING 1*) gene that encodes a cell-wall invertase required for carbon partitioning during early grain-filling (Wang et al. 2008). *GIF1* is responsible for grain weight reduction, ectopic expression of the cultivated *GIF1* gene with the 35S or rice Waxy promoter resulted in smaller grains, whereas over-expression of GIF1 driven by its native promoter increased grain production. These findings, suggest that *GIF1* is a potential domestication gene and that such a domestication-selected gene can be used for further crop improvement.

Establishing a reliable barley transformation technology is very important for the functional characterization of candidate genes and and the produced transgenic lines are subject for further studies.

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Molecular basis of the blood-brain barrier function

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One of the most important functions of the mammalian blood-brain barrier (BBB) is to restrict the free movement of different substances between blood and neural tissue, and it plays a key role in the homeostasis of the central nervous system. The principal components of the BBB are the cerebral endothelial cells that form a continuous monolayer and are interconnected with tight junctions and adherens

junctions. The tight junctions are composed of transmembrane proteins (occludins, claudins, junctional adhesion molecules) connected to junctional plaque proteins (*i.e.* ZO-1). The transmembrane proteins of the adherens junctions are the cadherins linked through catenins (alpha, beta, gamma) to the cytoskeleton.

The blood-brain barrier is involved in a large variety of pathological processes. Little is known about the effects of nicotine exposure on BBB function. We investigated the changes affecting the tight and adherens junction proteins by cigarette smoke components, especially nicotine and polyaromatic hydrocarbons (PAHs). 24h treatment of cerebral endothelial cells with relatively high concentration nicotine led to a decrease in occludin, cadherin and ZO-1 expression. Similar but less pronounced effects were observed after 24 h treatment with phenanthrene. Results of the immunofluorescent analysis confirmed western blot data. We also performed two dimensional electrophoresis in order to explore the cellular proteins responsive to nicotine and PAHs in brain endothelial cells. We observed different responses of the cells to both nicotine and phenanthrene treatment resulting in altered expression of shock induced proteins, metabolic enzymes, signaling molecules. This confirms the cerebral endothelium as being a target to cigarette smoke components (Hutamekalin et al. 2008).

From clinical point of view, because of the relative impermeability of the barrier many drugs are unable to reach the CNS in therapeutically relevant concentration, making the BBB one of the major impediments in the treatment of CNS disorders. A number of strategies have been developed to circumvent this problem. One of the successfully used methods to deliver drugs – especially antitumoral agents – to the CNS is the osmotic opening of the BBB using mannitol. This causes a rapid opening (within minutes) of the BBB which is reversible.

We investigated the effect of mannitol treatment on brain endothelial cells and found that mannitol induced a rapid, concentration dependent, and reversible tyrosine phosphorylation of a broad range of proteins between 50 and 190 kDa. One of the targets of tyrosine phosphorylation turned out to be the adherens junction protein beta-catenin and this phosphorylation was Src-kinase dependent (Farkas et al. 2005).

Beside beta-catenin and Src kinase, we aimed to find new signaling pathways activated by hypertonicity in cerebral endothelial cells and identified the receptor tyrosine kinase Axl to become tyrosine phosphorylated in response to hyperosmotic mannitol. Besides activation, Axl was also cleaved in response to osmotic stress. Specific knockdown of Axl increased the rate of apoptosis in hyperosmotic mannitol-treated cells; therefore, we assume that activation of Axl may be a protective mechanism against hypertonicity-induced apoptosis. Our results identify Axl as an important element of osmotic stress-induced signalling. (Wilhelm et al. 2008).

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Investigation of the maturation of NiFe hydrogenases in *Thiocapsa roseopersicina*

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Our model organism, *Thiocapsa roseopersicina* BBS is an anaerobic, phototrophic purple sulfur bacterium. There are at least two membrane-bound (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe] hydrogenases in the cells. A typical [NiFe] hydrogenase is composed of a large and a small subunit. The large subunit harbors a specific NiFe catalytic metallocenter associated with CO and CN ligands (Volbeda et al. 1995.) The maturation of these complex enzymes require numerous accessory proteins. Most of these auxiliary genes were found using transposon mutagenesis, one of them was the *hupK* gene. (Maróti et al. 2003.) The product of this gene, the HupK protein is present only in organisms containing at least one membrane-bound [NiFe] hydrogenase enzyme.

The role of HupK is not known yet. In order to investigate the role of this protein, *ΔhupK* mutant strains were created, then the hydrogenase activities of the wild type and the mutant strains were compared. The results clearly showed that HupK protein is important for the formation of the functionally active membrane-bound hydrogenases, but not for the biosynthesis of the soluble enzyme. (Maróti et al. 2003.)

More detailed information can be obtained from biochemical experiments. Special expression vector was used to produce active, tagged HupK protein in homologous host. The tagged HupK protein was purified under mild conditions to retain all protein-protein interactions and the copurified proteins were analyzed by mass spectrometry. From cells grown under standard conditions, only one protein partner, namely the GroEL chaperonin could be fished out. The specific role of GroEL in the hydrogenase maturation is not likely, therefore alternative growth conditions were used to find the specific partners. Nickel starvation of the cells is supposed to result in the accumulation of the intermediates of the posttranslational process. Therefore, the tagged HupK protein was purified from such cells, however only one co-purifying band was observed: the PuhA protein, which is the H subunit of the photosynthetic reaction centre.