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Identification of common mechanistic principles in plant and mammalian nucleoside transport proteins (Torsten Möhlmann)

In humans nucleoside transporters have diverse functions through interaction with purinergic receptors. In addition, nucleoside anologs are used to treat cancer. The Möhlmann lab has identified the first nucleoside carriers from plants. These carriers belong structurally to the equilibrative nucleoside transporters (ENT) but surprisingly some function as concentrative transporters (by use of an existing proton gradient). In plants, ENT function was found to be critical for pollen development, seed germination and maintaining the overall nucleotide balance. The Möhlmann lab will conduct detailed structure/function analysis by site directed mutagenesis and subsequent recombinant expression experiments. Having James Young, a world leading expert on nucleoside transporters, on the Canadian side optimal preconditions for these comparative approaches are given. AtENT1 shares only limited homology (26-28% identical amino acids) with human ENT proteins.
However, some amino acid residues identified to be critical for transport function and nucleoside or nucleoside inhibitor binding in human ENT proteins are conserved in AtENT1 (e.g. I33 (hENT2), L442 (hENT1), GxxxG motiv at pos. 441-445 (hENT1)). Initially, these positions will be mutated in AtENT1 and after expression in yeast cells the transport characteristics of the mutants will be analyzed. Human ENT3 has been shown to reside in internal (lysosomal) vesicular membranes. Structurally, hENT3 is characterized by a long N-terminal hydrophilic domain containing an acid di-leucine motive, which was found to be responsible for targeting to the lysosomal endomembrane.
Similarly, AtENT1 is characterized by an N-terminal extension compared to other Arabidopsis ENTs and harbours an acid di-leucine motive within that extension. Recently, we identified AtENT1 in the membrane of the vacuole. Therefore in a second project the role of the N-terminal extension and the identified motive for targeting of AtENT1 will be analyzed. Furthermore, we aim to retrieve putative interaction partners at this site by molecular approaches like split ubiquitin and direct interaction of tagged N-terminal AtENT1 polypeptides with plant protein extracts and subsequent purification of bound protein and mass spectrometric analysis. In this project we will interact with Eckhard Friauf who has expertise and equipment in mass spectrometric protein analysis. In addition, efforts to crystallize a first ENT protein (one member from the Arabidopsis protein family) will be undertaken in close cooperation with the Neuhaus group in Kaiserslautern. So far this has not been achieved for any ENT type protein. All necessary equipment for large scale protein production, purification and further processing is available in Kaiserslautern. A further, recent research interest of the Möhlmann group is connected to plant purine nucleoside catabolism. This metabolic pathway is highly compartmented in plants but the corresponding transport proteins (e.g. urate transporter) are still unknown. The Cheeseman group is working very successful on the aspect of urate transport in humans and thus represents an ideal cooperation partner in this project. It is planned to perform complementation approaches and to serach for candidate proteins in corresponding proteomes (e.g. peroxisome).