Heterologous expression of amino acid permeases from the vacuolar membrane of *Saccharomyces cerevisiae*

This work is part of a larger project aimed at functional and structural characterization of amino acid permeases (AAPs) from *Saccharomyces cerevisiae*. The end goal is the engineering of yeast strains for the excretion of amino acids.

Yeast are able to import a range of amino acids from their environment and also traffic them between the cytoplasm and the vacuole. Two groups of vacuolar amino acid transporters have been identified in *S. cerevisiae*: the amino acid vacuolar transport (AVT) family[1] and the vacuolar basic amino acid transporter (VBA) family[2]. These two groups are structurally and functionally distinct both from each other and from the better-characterized plasma membrane AAPs. To date they have been studied only in whole cells and isolated yeast vacuoles. Overexpression and purification of these proteins would allow for more detailed functional analyses, as well as be the first step towards obtaining a crystal structure.

Previous attempts to overexpress proteins of the VBA and AVT families in *Saccharomyces cerevisiae* were unsuccessful. However, it has been shown that overexpression in the heterologous yeast *Pichia pastoris* can provide improved yield and localization for *S. cerevisiae* plasma membrane AAPs. The aim of this student project is therefore to screen *P. pastoris* as an overexpression system for vacuolar amino acid permeases from *S. cerevisiae.*

1. Russnak, R. (2001). A Family of Yeast Proteins Mediating Bidirectional Vacuolar Amino Acid Transport. Journal of Biological Chemistry, 276(26), 23849–23857.

2. Shimazu, M. (2004). A Family of Basic Amino Acid Transporters of the Vacuolar Membrane from *Saccharomyces cerevisiae*. Journal of Biological Chemistry, 280(6), 4851–4857.

Primary goals

- Construction of pPICZ plasmids containing GFP- and His-tagged VBA1, VBA2, VBA3, VBA4 and VBA5 from *Saccharomyces cerevisiae*
- Chromosomal integration of these genes into *Pichia pastoris*
- Small-scale expression screening
- Determining localization of expressed genes

Secondary goals (dependent on time/success)

- *In vivo* functional assays
- Large-scale expression
- Purification trials

Techniques used

Primary

- USER cloning (including PCR, USER treatment, chemical transformation of *E. coli*, plasmid extraction, restriction-based analysis, sequencing)
- Yeast cell culture and electrotransformation
- MeOH-induced heterologous protein expression in *Pichia pastoris*
- Cell lysis and extraction of membrane fractions
- Analysis of protein samples by SDS-PAGE, in-gel fluorescence measurements, and Western blotting
- Confocal microscopy, including fluorescence-based imaging

Secondary

- Radioisotope-based amino acid uptake assays using whole cells
- Large-scale expression using a fermentor system
- Purification of membrane proteins by detergent based solubilization, Ni-affinity chromatography and size-exclusion chromatography