Functional analysis of the aquaglyceroporin Fps1p

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Abstract

Transport of compatible solutes is of vital importance in cellular osmoregulation. In the yeast *Saccharomyces cerevisiae* glycerol is accumulated under conditions of high osmolarity to maintain turgor and is released upon a drop in osmolarity to prevent water inflow and cell rupture.

The mediator of glycerol efflux is the aquaglyceroporin Fps1p. Fps1p belongs to the family of aquaporins, membrane proteins transporting water and/or solutes. Fps1p differs from many of the other members of the aquaporin family in that the otherwise well conserved NPA-motifs in the channel-forming B- and E-loop are replaced by NPS and NLA respectively. In this thesis Fps1p is shown to be functional with either or both NPA-motifs restored, indicating its similarities to the rest of the aquaporin family.

Fps1p is a regulated channel. Fps1p mediated transport is low during hyperosmotic stress to retain glycerol in the cell. Under hypo-osmotic conditions Fps1p becomes rapidly activated to facilitate the export of glycerol. Fps1p has very large, unique N- and C-terminal extensions. A part of the large N-terminal domain, close to the first transmembrane region, had previously been identified as restricting glycerol transport. In this work the region was further defined. Single amino acid changes in this domain caused similar effects as deletion of the whole region. The domain can be modelled to resemble the structure of the B- and E-loop of GlpF, the glycerol facilitator from E. coli, suggesting that also this region can dip into the membrane as they do.

The C-terminus is shown to be involved in the restriction of glycerol too and a regulatory domain somewhat similar to that in the N-terminus has been identified.

A random genetic screen for hyperactive mutants of Fps1p revealed mutations whose characteristics further strengthen the role of the N- and the C-termini to be involved in channel control and identified residues in the channel-forming B-loop.

A system was developed that allows testing substrate specificity of solute channels in yeast. Using this system we demonstrated how heterologous channels can be studied in yeast, how genetic structure/function analysis could be performed and how solute channels can be employed to study osmostress signal transduction.

Keywords: MIP, Fps1p, glycerol facilitator, aquaglyceroporin, osmoadaptation, regulation