Abstract

The aquaporins are transmembrane proteins belonging to an ancient and ubiquitous family. Aquaporins are present in all kingdoms of life and members of the family are commonly divided into two sub-categories. Orthodox aquaporins mediate rapid and highly selective flux of water, whereas aquaglyceroporins transport small neutral solutes that are important for specific processes. Thirteen different aquaporins have so far been identified in mammals. With their unique permeability characteristics and distribution in numerous tissues, the aquaporins have diverse roles in the regulation of water homeostasis. High resolution 3D structures are available for a few family members, which together with molecular dynamic simulations have uncovered the unique selective mechanism for free permeation of water or glycerol.

The present study is concerned with the yeast aquaglyceroporin Fps1 from *Saccharomyces cerevisiae*. Fps1 plays a central role in yeast osmoregulation, in which glycerol is accumulated under high osmolarity conditions to maintain turgor. Fps1 is a gated aquaglyceroporin, which restricts glycerol transport under hyperosmotic stress and rapidly mediates glycerol efflux in the adaptation to lower external osmolarity. A major goal is to understand the underlying mechanisms controlling Fps1 regulation. Compared to other aquaporins, Fps1 is exceptionally large due to long hydrophilic extensions in both termini. Defined stretches of twelve amino acids in each domain have been identified as crucial for proper channel regulation. Additional residues involved in channel control have been identified through truncation, site-directed, random and suppressor mutation analyses. To evaluate the effect of each mutation, Fps1 variants are expressed in yeast and characterised through *in vivo* assays. Structural modelling suggests that the N- and C-terminal regulatory domains dip into the membrane where they block the channel pore. Mutational analysis also support that the B-loop is involved in pore restriction. Recent data suggest that Hog1, a mitogen-activated protein kinase, is responsible for phosphorylation of the Fps1 N-terminus leading to channel closure.

The presence and conservation of putative aquaporin-encoding genes in fungal genomes has been investigated by comparative genomics. Fps1 orthologue sequences were found in nine yeast species and all have long hydrophilic extensions in common. The approach to overproduce and purify the 250 amino acid long Fps1 N-terminus for structural purposes, showed that this peptide is unstable and associated to other proteins. This suggests a possible role for the N-terminus in protein-protein interactions.

In summary, this thesis presents crucial domains and residues responsible for regulation, a role of Hog1 in controlling Fps1 and a purification protocol for an Fps1 soluble domain.

Key Words: Fps1, aquaporin, aquaglyceroporin, glycerol transport, osmoregulation, overproduction

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