Functional analysis of aquaporins using *Saccharomyces cerevisiae*

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Abstract: Aquaporins are membrane water channels present in all kinds of organisms, ranging from archaea to human. In mammals they are distributed in a tissue specific manner and play important roles in body water homeostasis. Aquaporins are involved in various diseases such as kidney disorders, tumor metastasis and brain edema, and are hence recognized as potential drug targets. The aquaporin family of proteins can be subdivided into orthodox aquaporins, transporting mainly water, and aquaglyceroporins, which additionally transport small uncharged compounds such as glycerol and urea. In this study the yeast *Saccharomyces cerevisiae* has been used for functional analyses of heterologous aquaporins as well as for elucidation of the roles of endogenous yeast aquaporins.

Expression of heterologous aquaporins (human AQP1, rat AQP3, 5 and 9 and *Plasmodium falciparum* PfAQP) in yeast results in osmosensitivity, due to water or glycerol leakage. Glycerol is the compatible solute employed by yeast cells. It is produced in response to osmotic stress and serves to regain cell turgor. Expression of aquaglyceroporins can suppress the osmosensitive phenotype of a strain unable to produce glycerol, if such cells are exposed to high levels of glycerol, demonstrating glycerol equilibration over the plasma membrane.

Water transport activity is demonstrated in a protoplast bursting experiment, in where protoplasts expressing orthodox aquaporins burst faster upon hypo-osmotic stress due to increased water inflow. Expression of aquaporins additionally has effects on osmotic signaling through the HOG pathway.

The genomes of fungi encode between one and five aquaporins, a number which differs even between relatively closely related species. This difference might indicate that aquaporins are under evolutionary pressure and are needed for specific lifestyles of certain yeast species.

The yeast *S. cerevisiae* has two orthodox aquaporins and two aquaglyceroporins. The orthodox yeast aquaporins are controlled at the transcriptional and possibly at the post-transcriptional level such that they are only expressed in a narrow window in the yeast life cycle. Aqy1 is produced in developing yeast spores where it probably plays a role in exporting water, perhaps in analogy to mammalian spermatogenesis. Aqy2 is expressed in actively proliferating cells, a situation that is not common for yeast in nature. It may play a role in rapid water movement during cell growth and expansion, but this remains to be investigated. The yeast aquaglyceroporin, Fps1, is gated by osmotic changes and play an important role in osmoregulation.

In summary, this thesis presents new assays to use for studying water and glycerol transport using yeast. In addition, new physiological roles of the endogenous yeast aquaporins are presented.

Key Words: aquaporin, water transport, glycerol transport, yeast, osmoregulation