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Osmoregulation at different stages of the yeast life cycle

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ABSTRACT

The ability to adapt to changing and potentially harmful conditions in the surrounding environment is crucial for fitness and survival of all living cells; in particular unicellular organisms, since they are frequently exposed to stress factors such as heat, drought, nutritional starvation and toxic substances. The aim of this thesis is to determine how cells respond to osmotic and nutritional changes in the environment and how downstream targets of signalling cascades are regulated.

Water is fundamental to life, and all cells must be able to adapt to fluctuations in water availability to maintain cellular water homeostasis. In bakers' yeast *Saccharomyces cerevisiae*, the High Osmolarity Glycerol (HOG) pathway is activated upon conditions of high osmolarity, and the pathway coordinates the responses needed to counteract loss of volume and turgor pressure. These actions include glycerol accumulation, ion efflux and transcriptional and translational changes. In this thesis, the osmotic stress response is characterized using a conditional osmotic system. We show that the period of Hog1 activation affects the transcriptional output in a quantitative rather than qualitative way. The analysis also sheds light on an initial adaptation process involving regain of volume through accumulation of compatible osmolytes, which precedes Hog1 nuclear accumulation and the transcriptional response.

The *S. cerevisiae* aquaglyceroporin Fps1 plays an important role during osmotic stress as a regulator of the intracellular glycerol concentration. A decrease in external osmolarity leads to water inflow and cell swelling, and Fps1 activity is vital under this condition for rapid release of excessive glycerol to lower the cells' turgor pressure. During a hyperosmotic shock, glycerol flux through Fps1 must be decreased; if not, the cells have great difficulties to accumulate glycerol and hence show osmosensitivity. The exact mechanisms behind Fps1regulation are still unknown, but regulatory domains on both cytoplasmic termini have been identified. Here, the importance of the Fps1 transmembrane core in restricting glycerol flux is described, and we show that the termini alone are not sufficient to regulate channel activity. We have also studied an orthodox aquaporin that is important for freeze and thaw resistance in the yeast *Pichia pastoris*. The activity of this aquaporin was shown to be regulated by a combination of phosphorylation and mechanosensitivity.

Finally, osmotic regulation throughout the yeast developmental pathways of sporulation and germination is briefly discussed. We have determined the transcriptional changes occurring during yeast spore germination and the analysis revealed a sequential upregulation of different subprograms that we can link to specific transcription factors. Although qualitatively similar responses, the transcriptional output of spores in response to glucose is not as pronounced as to rich growth medium, suggesting that spores can sense nutrient starvation early on in the quickening process.

Keywords: stress signalling, osmoregulation, aquaporins, cerevisiae, germination