Abstract

As water is a crucial component of all living systems, active osmoregulatory processes are of fundamental importance for biology. Like any other cell, the yeast *Saccharomyces cerevisiae* controls its internal water level to maintain biological processes.

At hyperosmotic conditions, i.e. following addition of salt to the growth medium, water rapidly flows out from the cell and its volume and turgor decrease. The yeast cell responds immediately to such an osmotic shock by activating the High Osmolarity Glycerol (HOG) signaling pathway. This conserved signaling system coordinates processes such as gene expression, translation and cell-cycle arrest which aim at adaptation to the new environmental condition and re-establishment of cellular water homeostasis. Particularly important is the increase of the intracellular glycerol levels, which is controlled by closing the glycerol export channel Fps1 and by increasing the production of the polyol. Glycerol accumulation drives water back into the cell, which then regains volume and turgor.

Osmoregulation involves a combined action of several processes, which eventually allows the yeast cell to adapt and to restart proliferation. In order to understand the relationships between different cellular events, such as signaling, gene transcription and cell volume regulation, we developed a mathematical model based on experimental data. The model reproduced experimental observations rather well, it could describe system properties, e.g. feedback control, and it had predictive value since simulations correctly forecasted the results of experiment which had not been done before. This thesis focuses on the biological interpretations that were used for model constructions as well as the conclusions drawn from computer simulations rather than on the mathematics employed to build the model

Following an osmotic shock, the transient activation of the HOG-pathway requires proper downregulation by feedback control mechanisms. This is crucial to relieve the cell cycle block and to resume proliferation. Pathway activation is severely prolonged in mutants defective in glycerol accumulation and shortened in cells with enhanced glycerol accumulation. Not glycerol *per se*, but rather accumulation of an osmolyte and cell reswelling seems to stimulate pathway downregulation. Simulations and experimental observations support the hypothesis that protein phosphatases consitutively deactivate Hog1 rather than actively determine the period of Hog1 phosphorylation. Changing the MAPK/phosphatase ratio can, however, alter signaling properties, such as amplitude and amplification of the signal.

The mathematical model constructed during this thesis will be further developed in order to improve its value as a tool for studying signaling properties. This will require more experimental data, especially at the level of single cells to better capture temporal and spatial events as well as cell-cell variations. We present a single-cell analysis method, employing microfluidics and optical tweezers, which allows a change of environment around the cell within a sub-second time frame. We show that this method allows measuring rapid volume changes and in the future it will be used to monitor protein movements and protein interactions in real time.

To date, the computational model only describes part of the signaling system, i.e. the Sln1-branch. In this thesis, pathway characteristics, such as Hog1 phosphorylation and gene expression, are compared between the Sln1 and the Sho1 branch, which together activate the pathway. The data suggest complex relationships. In the close future, we expect to include the Sho1-branch in the model and together with more experimental data we hope to unravel the individual contributions of the two branches to the overall response, a task that will require further close collaboration between the experimentlists and modelers.