UNIVERSITY OF GOTHENBURG

Stress, Homeostasis and Robustness: Molecular and Systems Level Analysis in Yeast

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Akademisk avhandling för filosofie doktorsexamen i mikrobiologi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras Torsdag den 24 Maj 2012, kl. 10.00 i föreläsningssal Ragnar Sandberg, Institutionen för kemi och molekylärbiologi, Medicinaregatan 7, Göteborg.

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Abstract

Cells constantly encounter stress due to alterations in the external milieu or internal parameters. However, cells are robust to such changes and maintain homeostasis. Using the yeast *Saccharomyces cerevisiae* as a model organism, we attempted to elucidate aspects of homeostasis and robustness at both molecular and systems levels. At the molecular level, we focused on the aquaglyceroporin Fps1 and at the systems level we investigated the High Osmolarity Glycerol (HOG) pathway. Although Fps1 plays a key role in osmotic regulation and homeostasis, the mechanisms controlling Fps1 are not yet fully understood. We present evidence that Hog1 restricts the flux of glycerol and arsenite through Fps1 by phosphorylation of a residue within the N-terminal regulatory domain. This is the first report of a Mitogen Activated Protein Kinase (MAPK) regulating an aquaglyceroporin. In addition, we demonstrate that yet another MAPK, Slt2 may also be involved in regulating Fps1. It appears that Slt2 controls Fps1 by stimulating Fps1-mediated efflux. A residue within the C-terminal regulatory domain seems to be involved in controlling arsenite efflux through Fps1. Moreover, we show that in addition to the N- and C- termini, the transmembrane core of the protein also has a large effect on the transport activity of Fps1. Taken together, we speculate that phosphorylation of the termini affects the orientation of the transmembrane helices, thereby disturbing the transport activity of the protein. At the systems level we challenged the yeast HOG signal transduction pathway with systematic perturbation in the expression levels of its components under various external conditions to identify fragile nodes. We observed a high frequency of fragile nodes within the HOG pathway due to the inherent nature of signal transduction and the distribution of these fragile nodes was independent of function or location in the pathway topology. Moreover, the fragility patterns were mainly independent of the overall pathway activation status in response to different stresses. We found that the toxicity upon overexpression is at least partly due to pathway hyperactivation and can be suppressed by deletion of upstream or downstream pathway components. Furthermore, *in silico* analysis highlighted the impact of model structure on *in silico* robustness, and suggested complex formation and scaffolding as important contributors to the observed fragility patterns. Collectively, we believe that the robustness analysis can provide complementary information to dynamic data improving understanding of the operation of cellular signalling networks.

Keywords: Stress, signalling, homeostasis, robustness, osmohomeostasis, Fps1, HOG pathway, *Saccharomyces cerevisiae*

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