

Probing Osmo-adaptation Kinetics in *Saccharomyces cerevisiae* with Periodic Stimuli

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Reactions in signaling cascades operate over a wide variety of timescales ranging from rapid ligand-protein interactions to slower downstream gene expression. It is often not clear which pathway components and reactions dominate cascade output dynamics, especially in response to dynamic input signals. To learn how signals are processed in a MAPK cascade, we use periodic stimuli to experimentally measure the frequency dependence of the osmo-adaptation pathway in *Saccharomyces cerevisiae*. This network contains multiple feedback loops, which act to maintain intracellular osmolyte concentrations in response to changes in the extracellular osmolarity. These feedbacks utilize diverse mechanisms such as protein-protein interactions, gene expression, metabolic activity and membrane channel activation. We apply system identification methods from control engineering and infer a concise predictive model of the dynamics of the MAPK Hog1, a key regulatory protein in the network. We then ask what possible model architectures can give rise to the signaling dynamics observed experimentally. By comparing this network architecture with known genetic and biochemical data, we find that the dynamics of osmo-adaptation is dominated by a fast-acting negative feedback through the kinase Hog1 that does not require new protein synthesis. At elevated osmo-shocks an additional, much slower, negative feedback acting through gene expression allows cells to adapt faster to future stimuli. This analysis sheds light on how the multiple feedback structure of the osmo-adaptation network allows cells to respond rapidly and robustly to fluctuating extracellular environments. Further, this work serves as proof of principle for studying the link between signal processing and network architecture in cell biology.