Robust network structure of the Sln1-Ypd1-Ssk1 three-component phosphorelay prevents unintended activation of the HOG MAPK pathway in *Saccharomyces cerevisiae*

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***Short Abstract* —Under normal growth conditions a three-component (Sln1-Ypd1-Ssk1) phosphorelay represses high-osmolarity glycerol (HOG) pathway activity in the yeast *Saccharomyces cerevisiae*. This inhibition is essential for cellular fitness in normal osmolarity. We established that the phosphorelay is robust and maintains inhibition of the HOG pathway even after significant changes in the levels of its three components. A biochemically realistic mathematical model of the phosphorelay suggested that robustness is due to buffering by a large excess pool of Ypd1. We confirmed this result experimentally. Buffering by an intermediate component in excess represents a novel mechanism through which a phosphorelay can achieve robustness.**

***Keywords* — robustness, HOG pathway, osmotic stress, histidine kinase, mathematical modeling, invariants.**

I. Overview

DESPITE its importance during periods of increased osmolarity, unintended activation of the high-osmolarity glycerol (HOG) pathway during growth in normal osmolarity conditions is severely deleterious to the budding yeast *Saccharomyces cerevisiae* [1]. The Sln1-Ypd1-Ssk1 three-component phosphorelay is responsible for maintaining inactivation of the HOG pathway. Under normal osmolarity the phosphorelay is active and maintains Ssk1 in its phosphorylated form. In response to osmotic shock, the phosphorelay is inactivated, and Ssk1 is rapidly dephosphorylated. Unphosphorylated Ssk1 then activates downstream HOG pathway components. It is thus the essential controller of HOG pathway activity.

We undertook a comprehensive characterization of the sensitivity of HOG pathway activation to changes in the expression levels of the phosphorelay proteins Sln1, Ypd1, and Ssk1. We systematically under- and overexpressed the three proteins using an artificial induction system and found that phosphorelay activity is robust to changes in the concentrations of its components. We developed a detailed, biochemically realistic mathematical model of the HOG pathway three-component phosphorelay to elucidate the mechanism underlying this robustness. Our model incorporates extensive structural and mechanistic information about the phosphorelay and considers nearly all possible interactions between the three relay proteins. We used mass-action kinetics and algebraic calculations to characterize the steady-state behavior of the model. Steady-state algebraic models are a useful alternative to existing computational models of the HOG pathway for understanding robust behavior. Algebraic manipulations can be done without ever assigning special values to the parameters (i.e., the rate constants in the reaction network), many of which are difficult or impossible to measure experimentally [2].

Our steady-state analysis predicted that relative levels of dephosphorylated Ssk1 depend solely on Ypd1 levels and that robustness is achieved by maintaining Ypd1 in large excess. We experimentally tested this prediction by perturbing protein expression levels so as to deplete this buffering pool of Ypd1. All such perturbations compromised the ability of the phosphorelay to inhibit the HOG pathway, leading to hyperactivation in normal osmolarity conditions. The presence of a large buffering pool of an intermediate phosphorelay component is a previously underappreciated mechanism for robustness and suggests a possible advantage of a three-component relay over a two-component system.

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