Genetic control of robustness and tunability in the yeast osmosensing signaling pathway

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***Short Abstract* — Genetic variation underlies much of the phenotypic diversity in nature, but the robustness of cellular networks to coding sequence variations is often difficult to quantify. Here, we employed the high osmolarity glycerol (HOG) pathway in *S. cerevisiae*. By performing systematic orthologous pathway gene substitutions and time-lapse microscopy, we found that signaling was significantly altered by sequence variations in the downstream MAPK genes, but remained relatively robust to changes in the upstream phosphorelay components. From a computational robustness analysis, we found that signaling is most sensitive to kinetic parameter changes involving the MAPK cascade. We then performed evolution experiments on yeast cells with hyperactive HOG signaling. Strikingly, these cells rapidly restored wild-type fitness and signaling mainly via point mutations in the MAPK genes. Our results suggest that the skewed sensitivities of signaling dynamics to underlying component variations is a direct consequence of its biochemical circuitry, and might impact the evolvability of this network.**

I. Purpose

REMARKABLY, organisms can exhibit phenotypic robustness against a diverse array of stochastic, environmental and genetic variation. Studies on the robustness of cellular phenotypes to gene expression changes have been greatly facilitated by experimental techniques which allow quantitative manipulation of gene expression [1-2]. In contrast, there is no simple method to comprehensively assess the robustness of network function to coding sequence variation of its component genes.

To address this, we take a three-pronged strategy combining experimental, computational and evolutionary approaches to investigate the robustness of cellular signaling to genetic perturbations of its underlying molecular network. We employ the well-characterized high osmolarity glycerol (HOG) pathway in the budding yeast *S. cerevisiae*, which forms a core module of the hyperosmotic shock response [3].

The HOG pathway consists of a phosphorelay chain of proteins (Sln1, Ypd1 and Ssk1) that acts on a downstream MAPK cascade (Ssk2, Pbs2 and Hog1) to ultimately modulate Hog1 activity [3]. Upon activation, Hog1 translocates into the nucleus to initiate transcriptional changes in response to the osmotic shock [3].

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II. Results

A. HOG signaling displays varied sensitivity to ortholog substitutions

HOG signaling dynamics of the Sln1- and Ypd1-ortholog (from *C. glabrata* and *C. albicans*) hybrid pathways were indistinguishable from that of the wild-type *S. cerevisiae* response upon a hyperosmotic shock. But the majority of Ssk2- and Pbs2-ortholog hybrid pathways displayed grossly defective signaling.

B. Computational analysis predicts HOG signaling is most sensitive to MAPK cascade parameter variations

Key dynamical properties of HOG signaling (peak Hog1 phosphorylation level and the initial Hog1 phosphorylation rate) remained almost unchanged upon varying Sln1- and Ypd1-associated parameters over a wide range of parameter space. But MAPK-genes-associated parameter changes significantly altered the signaling output landscapes.

C. PBS2 and SSK2 mutations found in independent evolution experiments are mainly responsible for tuning of signaling dynamics and improved fitness

We induced hyperactive HOG signaling in yeast cells [4], and harnessed evolution and natural selection to identify adaptive genetic variants that can significantly down-regulate signaling, and thus restore fitness. Mutations in the MAPK cascade genes (*PBS2* and *SSK2*) dominate the genetic changes among the pathway genes across the majority of the independently adapted populations, consistent with the computational robustness analysis.

References

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