Mechanisms for ultrasensitivity, response time and robustness in the galactose genetic switch

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The galactose-signaling pathway in *Saccharomyces cerevisae* transforms small variations in extracellular galactose into an all-or-none response. We show that the promoter activity of the galactokinase, GAL1 is ultrasensitive to changes in concentration of the signal transducer, Gal3p. Experiments and mathematical modeling reveal that in the absence of feedback the abundance of the repressor (Gal80p) controls the threshold of activation and the degree of ultrasensitivity. Parameter sensitivity analysis suggests that a combination of mechanisms contribute to ultrasensitivity. We compare a diverse set of galactose-regulated promoters that differ in the number and spacing of GAL4p binding sites and demonstrate that multiple binding sites modify the dynamic range but not the cooperativity. We demonstrate that the nested feedback loops provide robustness to parameter variations. Finally, a steady-state analysis demonstrates that two stages of sequestration generates larger ultrasensitivity and may possess an additional feature of decreasing the response time.

Keywords **— Ultrasensitivity | galactose network | molecular sequestration | logarithmic sensitivity**

I. Introduction

The galactose network in *Saccharomyces cerevisae* is a tightly regulated metabolic switch that is activated in the presence of galactose. Extensive molecular characterization of the network has not identified the dominant mechanisms that contribute to ultrasensitivity, response time and robustness to parameter variations.

Nonlinear input-output responses can be produced by a diverse set of molecular mechanisms including zero-order kinetics, positive feedback, cooperativity and molecular sequestration [1-3]. Sequestration of an activator by an inhibitor produces ultrasensitivity for specific ranges of the dissociation constant (K_d) and inhibitor concentration.

II. Results

We measured the maximal logarithmic sensitivity, $S_L = \max\left(\frac{d\log(Output)}{d\log(Input)}\right)$, in synthetic galactose circuits between Gal3p (input) and P_{GAL1}-YFP (output) in the presence and absence negative feedback. In the absence of feedback, varying the ratio of Gal80p to Gal3p determines the activation threshold and degree of ultrasensitivity at steady-state, suggesting that sequestration between Gal80p and Gal3p is an important mechanism for producing ultrasensitivity. Mathematical modeling and parameter sensitivity analysis suggests dual levels of sequestration (Gal80p-Gal3p and Gal80p-Gal4p) are critical for an ultrasensitive response.

Galactose-regulated promoters vary in the number and spacing of GAL4 binding sites. To determine whether multiple GAL4 binding sites contribute to the ultrasensitivity response, we measure the transfer functions of all GAL4 regulated promoters (GAL 1, 2, 3, 7, 10, 80, GCY1) and each individual GAL4 binding site using a several approaches. These data show that multiple GAL4 binding sites increase the dynamic range, but do not alter the cooperativity since all promoters displayed a Hill coefficient of ≈1.5-2. The steady-state promoter activities of GAL2 (permease), GAL3 and GAL80 provide the relative strengths of the feedback loops.

Further, we demonstrate that a two-stage sequestration cascade produces larger ultrasensitive responses when the binding and dissociation rates are equivalent compared to a single-stage mechanism. A two-level cascade can respond faster to changes in stimuli due to larger off-rates than a one-stage at constant S_L. Multi-stage sequestration is prevalent in natural networks [4-5]. Based on these findings, we hypothesize dual sequestration may have been selected by evolution to enhance the response time to changes in nutrient conditions.

III. Conclusions

Experiments and mathematical modeling identify mechanisms that contribute to ultrasensitivity in the galactose network. Our results suggest a tradeoff between the length of a sequestration cascade and the response time.

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