Perturbation-Based Analysis and Modeling of Combinatorial Regulation in the Yeast Sulfur Assimilation Pathway

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IN yeast, the pathways of sulfur assimilation are combinatorially controlled by five transcriptional regulators (three DNA binding proteins: Met31p, Met32p, and Cbf1p; and two cofactors: Met4p and Met28p) and a ubiquitin ligase subunit (Met30p) with specificity for Met4p. This regulatory system exerts combinatorial control not only over sulfur assimilation and methionine biosynthesis, but also many other physiological functions in the cell. The ways in which the complexity of the control system relates to the diversity of regulated functions is only partially understood, largely because the methods that have been applied cannot reliably distinguish direct from indirect actions of individual transcription regulators. Recently, we characterized a gene induction system, which, upon the addition of an inducer, results in near-immediate transcription of a gene of interest under physiological conditions. We used this to perturb steady state growth in chemostats, which facilitated distinction of direct from indirect effects of individual factors dynamically by quantitation of the subsequent changes in genome-wide patterns of gene expression. The patterns that emerged from this analysis of the methionine regulators delineate distinct and overlapping transcriptional regulatory niches for each of the factors. We discovered and quantitatively modeled feedback relationships among the regulators, notably feed-forward regulation of Met32p (but not Met31p) by Met4p that generates dynamically differences in abundance that can account for the differences in function of these two proteins despite their identical binding sites. We were able to show directly that Cbf1p acts sometimes as a repressor and sometimes as an activator. At promoters responsive to the Cbf1p-Met28p-Met4p complex Cbf1p activates immediately after induction, and then, over time, becomes a repressor, probably because it's concentration exceeds those of its cofactors. Combining these results with those obtained from our studies of deletion mutants of the same regulators we can derive a reasonably comprehensive picture of combinatorial regulation of all the diverse physiology under combinatorial control by the Met pathway regulators.