Systems-Level Analysis of Mechanisms Regulating Yeast Metabolic Flux

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Project Goals: To apply the integrated power of modern 'omic technologies to identify physiologically relevant metabolic regulation in a systematic, large-scale, and quantitative manner in Baker's yeast and (going forward) also Clostridia.

INTRODUCTION: Metabolism is among the most strongly conserved processes across all domains of life and is crucial for both bioengineering and disease research, yet we still have an unclear understanding of how metabolic fluxes are determined. Qualitatively, this deficiency involves missing knowledge of enzyme regulators. Quantitatively, it involves limited understanding of the relative contributions of enzyme and metabolite concentrations in controlling flux across physiological states. Addressing these gaps has been challenging because in vitro biochemical approaches lack the physiological context, whereas models of cellular metabolic dynamics have limited capacity for identifying or quantitating specific regulatory events because of model complexity.

RATIONALE: Flux through individual metabolic reactions is directly determined by the concentrations of enzyme, substrates, products, and any allosteric regulators, as captured quantitatively by Michaelis-Menten kinetics. Experimental variation of reaction species in vitro allows for the inference of regulators and reaction equation kinetic parameters. Analogously, physiological changes in flux entail a change in reaction species that can be used to determine reaction equations based on cellular data. This requires measurement across multiple biological conditions of flux, enzyme concentrations, and metabolite concentrations. We reasoned that chemostat cultures could be used to induce predictable and strong flux changes, with changes in enzymes and metabolites measurable by proteomics and metabolomics. By directly relating cellular flux to the chemical species that determine it, we can carry out regulatory inference at the level of single metabolic reactions using cellular data. An important benefit is that the physiological significance of any identified regulator is implicit from its role in determining cellular flux.

RESULTS: Here we describe an approach for *Systematic Identification of Meaningful Metabolic Enzyme Regulation* (SIMMER). We measured fluxes, and metabolite and enzyme concentrations, in each of 25 yeast chemostats. For each of 56 reactions for which the flux, enzyme, and substrates were measured, we determined whether variation in measured flux could be explained by simple Michaelis-Menten kinetics. We also evaluated alternative models of each reaction's kinetics that included a suite of allosteric regulators drawn from across all organisms. For 46 reactions, we were able to identify a useful kinetic model, with 17 reactions not including any regulation and 29 reactions being regulated by one to two allosteric effectors. Three previously unrecognized cross-pathway regulatory interactions were validated biochemically. These included inhibition of pyruvate kinase by citrate, which accumulated and thereby curtailed glycolytic outflow in nitrogen-limited yeast. For well-supported reaction forms, we were able to determine the extent to which nutrient-based changes in flux were determined by changes in the concentrations of individual reactants, products, enzymes, or allosteric effectors. Overall, substrate concentrations were the strongest driver of the net rates of cellular metabolic reactions, with metabolite concentrations collectively having more than double the physiological impact of enzymes.

CONCLUSION: By connecting changes in flux to their root cause, SIMMER parallels classic in vitro approaches, but it allows testing under physiological conditions of numerous regulators of many reactions simultaneously. Its application to yeast showed that changes in flux across nutrient conditions are predominantly due to metabolite, not enzyme, levels. Thus, yeast metabolism is substantially self-regulating.

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