Integration of carbon, nitrogen, and oxygen metabolism in *Escherichia coli*

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Project Goals:

A key challenge for living systems is balancing utilization of different elemental nutrients, such as carbon, nitrogen, and oxygen. Although the regulation of specific nutrient assimilation systems has been extensively studied, how these systems coordinate with each other remains poorly understood. Here we aim to obtain a quantitative understanding of such coordination in Escherichia coli with a focus on fast-acting regulatory mechanisms. To this end we are (1) quantifying metabolic responses to nutrient perturbations using metabolomics, (2) building differential equation models that bridge multiple nutrient systems, and (3) discovering regulatory principles from this unified combination of metabolomics and modeling.

Results:

Biomass production requires the integration of multiple external nutrients, whose availability is subject to environmental fluctuations. As growth can be limited by the scarcity of any one nutrient, the rate at which each nutrient is assimilated must be sensitive not only to its own availability, but also to that of other nutrients. Remarkably, across diverse nutrient conditions, *E. coli* grow nearly optimally: they balance effectively the conversion of carbon into energy versus biomass, and excrete only small amounts of waste.

What type of regulatory architecture might (i) enable homeostasis of intracellular metabolite concentrations and (ii) produce metabolic fluxes that nearly optimize growth? The most basic homeostatic regulatory mechanism is feedback inhibition. Through a series of reductionist differential equation models, with fluxes expressed in Michaelis-Menten form, we found that *feedback inhibition alone is sufficient to meet both of the above goals*. While such a result was expected for a linear biosynthetic pathway, it was striking that feedback inhibition can in theory also effectively regulate metabolic cycles, the portioning of carbon between energy production and biomass, and the integration of multiple nutrients (e.g., nitrogen and carbon).

We then considered how actual feedback regulatory architectures compare with those predicted to be effective based on our conceptual analysis. *E. coli's* ammonia assimilation cycle, involving the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT), is among the best-studied metabolic cycles from the perspective of regulation. Despite this, existing literature focuses on a single feedback (from glutamine on GS), whereas our analysis indicated the need also for a feedback from glutamate on GOGAT. Through detailed metabolomic analysis and modeling of the cycle, including analysis of various metabolic and regulatory mutants, we showed that a feedback on GOGAT is indeed required, and that surprisingly it is provided by aspartate, whose concentration mirrors that of glutamate.

To investigate the link between carbon and nitrogen metabolism, we measured changes in the glucose uptake rate in response to changing nitrogen availability. We found that glucose uptake in *E. coli* is subject to rapid

regulation by nitrogen. Remarkably, nitrogen up-shift doubles glycolytic flux in 2 min without substantial changes in the concentration of any glycolytic intermediate. What type of feedback might enable such a response? The most elegant possibility involved the carbonaceous substrate of nitrogen assimilation *α-ketoglutarate directly inhibiting the phosphotransferase system,* which simultaneously uptakes glucose and drains the bottom of the glycolysis pathway. Such a feedback loop, never previously proposed, was verified biochemically and shown genetically to be both *necessary and sufficient for nitrogen-based regulation of sugar uptake*.

Much of central carbon metabolic flux is altered by changing oxygen levels. Nevertheless, there are parallels to the case of nitrogen metabolism: α-ketoglutarate, the product of carbon metabolism that directly feeds nitrogen metabolism, controls carbon metabolic response to nitrogen availability; similarly, we believe that NADH, the product of carbon metabolism that immediately feeds into oxygen metabolism, controls carbon metabolic response to oxygen availability. NADH levels impact carbon metabolism in multiple ways, including shutting off the right side of the tricarboxylic acid (TCA) cycle, reversing the left side of the TCA cycle, and inhibiting glycolysis. This is basically feedback inhibition writ large.

Finally, we considered when feedback inhibition might be insufficient as a metabolic regulatory strategy. Our analyses suggested its sufficiency for all steady-state conditions. Therefore, we considered the possible importance of other regulatory motifs in oscillating conditions. A proposed feed-forward circuit involves activation of phosphoenolpyruvate carboxylase (the anapleurotic enzyme in *E. coli* that provides new 4 carbon units into the TCA cycle) by fructose-1,6-bisphosphate. We verified genetically the existence of this regulation, found that the regulation is ultrasensitive, and showed that ultrasensitive activation of this enzyme by fructose-1,6-bisphosphate markedly enhances *E. coli* growth specifically during oscillating glucose conditions.

Taken together, these findings support a general mechanism for nutrient integration: *Limitation for a nutrient other than carbon leads to build-up of the most closely related product of carbon metabolism, which in turn feedback inhibits further carbon metabolism.* Our efforts to translate this principle into a predictive, quantitative model of central carbon, nitrogen, and oxygen metabolism are ongoing. Eventual success will require intensive re-examination of the regulation of many enzymes, with the ultimate payoff being enhanced understanding and rational engineering of bacterial metabolism.