Robust Control in the Nitrogen Assimilation System of E.coli

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Short Abstract **— Bacteria simultaneously regulate the assimilation of multiple nutrients to enable optimized growth. How this precise balance is maintained, despite fluctuations in metabolic sources and sinks in the cell, and variations in concentrations of enzymes that make up the regulatory circuits? Here we address this by studying the nitrogen assimilation system of *E. coli*. A novel mechanism which is based on avidity of a bifunctional enzyme to its multimeric substrate is shown to provide a robust balance between carbon and nitrogen pools in the cell. We find experimentally that a robust ratio between the two pools is maintained and that this robustness carries through to the cells growth rate.**

Keywords **— Avidity, GS, AT/AR, bifunctional enzymes.**

I. Introduction

E. *coli* uses a fascinating system to regulate nitrogen assimilation. The key enzyme in this system, glutamine synthetase (GS), produces glutamine from glutamate and ammonia [1]. GS has an elaborate control mechanism with several unusual biochemical features. First, GS is active as a dodecamer made of 12 identical monomers. The activity of GS is determined by a reversible covalent modification: each monomer can be either adenylated or unadenylated, with adenylation inactivating the monomer's activity. The total activity of GS is the sum of its active monomers, without discernable cooperative effects [2].

A second feature of this system is that the modification of GS is carried out by a bifunctional enzyme that catalyzes two opposing reactions. This enzyme, called adenylyl-transferase/adenylyl-removing enzyme, AT/AR, both adds and removes the adenyl modification of GS, using two distinct domains [3]. The rates of these opposing reactions depend on two inputs: glutamine and the TCA-cycle metabolite α-ketoglutarate, which is the carbon backbone for glutamate and glutamine [4].

By responding to both glutamine and its carbon backbone, α-ketoglutarate, the nitrogen system effectively senses the ratio between nitrogen and carbon: nitrogen is represented by glutamine and carbon by the TCA metabolite α-ketoglutarate. While the ratio of glutamine to α-ketoglutarate varies dramatically in response to nitrogen limitation, this ratio is remarkably steady under other conditions, such as carbon limitation [5]. Such a steady ratio requires tuning the rate of ammonia assimilation to match precisely the carbon uptake rate, to avoid draining the carbon pool of the cell by producing too much glutamine.

II. Results

We use theory and experiment to ask whether the unusual biochemical details of the nitrogen system have a functional meaning. Specifically, why does this system use a bifunctional enzyme that catalyzes two opposing reactions and why is GS multimeric? We find that these biochemical features can work together to provide a systems-level function: robustness of the ratio of α-ketoglutarate to glutamine in the face of wide variations in the levels of all of the proteins in the core circuit, and in the metabolic inputs such as ammonia and carbon source. The ratio is robust also to variations in the cells demand for glutamine. This robustness means that the circuit can function properly to balance carbon and nitrogen fluxes despite the noise inherent in the proteins and environment. The essence of the mechanism is based on avidity of the bifunctional enzyme AT/AR to adjacent monomers of GS on the same dodecamer.

To test this model we used genetic perturbations, controlled gene expression and mass-spectrometry of metabolite levels. We find that the ratio of glutamine to α-ketoglutarate is indeed robust to wide variations in the level of GS, and that this robustness depends on the bifunctional enzyme AT/AR. We further find that robustness carries through to the growth rates of the cells: growth rate is robust to wide variations in the levels of GS and this robustness depends on AT/AR. Moreover, addition of a mono-functional AT/AR mutant in the presence of the native AT/AR enzyme breaks robustness, in accordance with the model predictions.

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