Coping with noisy metabolism in the bacterial microenvironment

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The cellular microenvironment is dynamic, which fluctuations in nutritional resources and cellular protein composition. Eukaryotes cope with this noise through enzyme regulation, such as enzyme post-translational modification (PTMs). However, for decades it has been asserted that few prokaryotic enzymes are regulated by PTMs. Recent proteomic studies challenge this assumption, having discovered many PTMs on prokaryotic metabolic enzymes. To elucidate the biochemical functions of these PTMs and their influence on *E. coli* physiology, we developed an approach that integrates proteomic data, metabolic network analysis, protein structure analysis, and targeted genome engineering. Using this, we demonstrate that many PTMs aid in rapidly regulating metabolism to cope with fluctuations in the nutritional microenvironment of the cell. Specifically, we use a novel metabolic pathway modeling method, called Regulated Metabolic Branch Analysis (RuMBA), to identify enzymes that should require metabolic regulation in response to noise from fluctuating metabolite concentrations. We show that PTMs are particularly enriched among these enzymes and complement known allosteric regulatory mechanisms. Furthermore, regulated PTM sites are highly conserved and located near enzyme active sites. We further elucidate detailed mechanisms by which these PTMs regulate flux by integrating RuMBA with enzyme assays, protein structure analyses, and screens of PTM mutants generated by multiplexed automated genome editing technologies. Through this we show that PTMs are employed far more than previously anticipated to regulate prokaryotic metabolism in response to intrinsic and extrinsic metabolic noise.

Transcriptional vs post-transcriptional regulation of the central carbon metabolism of *E. coli*

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Transcriptomics data are currently one of the most available types of large-scale biological data. A large number of methods have been developed to improve constraint-based simulations using these data. We recently performed a systematic comparison of these methods and observed that, at least for central carbon metabolism, there is no significant improvement in the prediction of flux distributions when gene expression data is used. These results are consistent with recent studies, in different organisms, showing that central carbon metabolism is predominantly regulated at post-transcriptional levels. Central carbon metabolism provides the precursors for the production of multiple compounds used in industrial biotechnology. Hence, it is the main target for intervention in most rational strain design strategies. However, its complexity is still not completely understood. In this work, we analyze the role of allosteric regulation, one of the main mechanisms of post-transcriptional regulation, for the control of central carbon metabolism. We extend a model of central carbon metabolism of *E. coli* with allosteric interactions, revealing a hidden topology in metabolic networks. We use this model to integrate a multi-omic dataset containing transcript, protein, flux and metabolite levels to further dissect the contribution of different types of regulation for metabolic flux control in these central pathways. Situations of predominant allosteric control could be identified, highlighting the importance of this kind of regulation in central carbon metabolism.