Collective genetic units in bacterial metabolism

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Metabolic enzymes must reliably catalyze the conversion of substrate to product, but are also constrained to function properly in the context of their pathway. Here we show that constraint on the accumulation of a metabolic intermediate leads to epistasis and co-evolution of two enzymes in folate metabolism: Dihydrofolate reductase (DHFR) and Thymidylate synthase (TYMS). These two enzymes co-evolve: (1) experimentally during forward evolution and (2) statistically across 1445 bacterial genome sequences. Moreover, these two enzymes evolve relatively independent from the remainder of the pathway. These results motivate the development of new co-evolutionary analyses for examining sequence constraints within metabolism.

***Keywords* — coevolution, statistical genomics, folate metabolism, dihydrofolate reductase (DHFR), epistasis, experimental evolution**

I. Introduction

In metabolism, the coordinated activity of multiple enzymes produces the substrates necessary for cell growth and division. Though a large body of prior work has elucidated the molecular components and biochemical reactions comprising central metabolism, it remains unclear what evolutionary and functional constraints act on metabolic enzymes. For example, if one enzyme in a pathway is inhibited or otherwise reduced in activity, what (if anything) has to happen in the rest of the pathway to compensate? And to what extent are the activities of metabolic enzymes coupled or entirely independent from one another? We chose folate metabolism, a well-characterized and highly conserved pathway, as a model system to study these questions. Our approach combines coevolutionary analyses, epistasis measurements, and experimental forward evolution in order to understand the evolutionary constraints on this system.

II. Results

We conducted an analysis of thirteen enzymes comprising the core one-carbon folate metabolic pathway across 1445 bacterial genomes. Two measures of coevolution were considered: 1) synteny, the conservation of chromosomal proximity between genes and 2) co-occurrence, the coordinated loss and gain of genes across species. The results indicate a sparse architecture of interactions, in which most genes evolve independently of one another, with several small groups that coevolve modularly.

Expectedly, one of these groups is composed of the glycine cleavage system proteins H, P and T, which make up a physical complex. The second coevolving unit is made up of two enzymes, dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), which catalyze sequential reactions but are not known to physically bind. We chose DHFR and TYMS for detailed experimental study in *E. coli*. Quantitative epistasis measurements reveal that DHFR and TYMS are coupled such that a decrease in the activity of one enzyme can be compensated for by lowering the activity of the other. In concordance with this, metabolomic measurements suggest that this epistasis is driven by a constraint on their relative activities, which must be balanced to prevent the accumulation of a toxic intermediate. We evolved wild-type *E. coli* in the presence of trimethoprim, a competitive inhibitor of DHFR. Whole genome sequencing reveals that resistance is obtained by mutations in both DHFR and TYMS, but not other genes in the pathway. Thus, the enzyme pair shows a capacity for adaptation that is independent from the rest of folate metabolism, which is supported by both statistical and experimental evidence.[2]

III. Discussion

This work suggests that complex systems such as folate metabolism may be subdivided into functional units that act collectively and adapt relatively independent of one another. We provide one such example, which was predicted by statistical analysis of genomic data. Our results motivate a global analysis of coevolution within metabolism, to be followed by comprehensive experimental testing. This strategy has the potential to provide important insights toward rationally engineering new systems, and predicting the combined effects of mutations.

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