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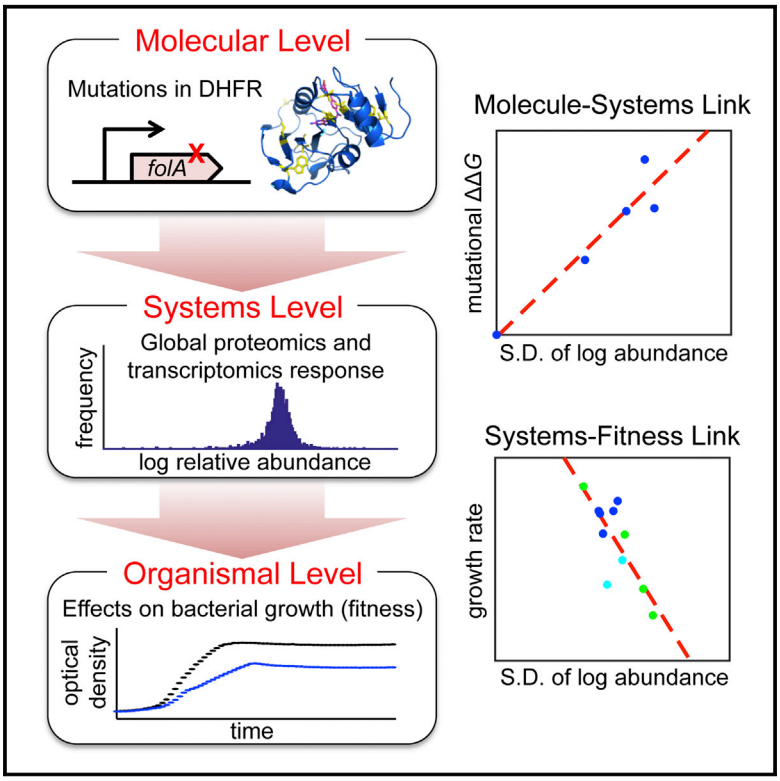
Systems-Level Response to Point Mutations in a Core Metabolic Enzyme Modulates Genotype-Phenotype Relationship

Graphical Abstract



Highlights

- Mutations in a metabolic enzyme DHFR induce a global and specific systems-level response
- Mutational changes in proteome are quantitatively linked to fitness
- Chromosomal mutations in DHFR have highly pleiotropic effect
- Several genes are overexpressed in mutants yet their protein abundance drops

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In Brief

Understanding the relation between protein function and organismal fitness is challenging due to sytems-wide pleiotropic effects. Bershtein et al. show that a global proteome and transcriptome response to destabilizing mutations in a core metabolic, enzyme dihydrofolate reductase (DHFR), quantitatively links the molecular effects of mutations to bacterial fitness.

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Systems-Level Response to Point Mutations in a Core Metabolic Enzyme Modulates Genotype-Phenotype Relationship

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SUMMARY

Linking the molecular effects of mutations to fitness is central to understanding evolutionary dynamics. Here, we establish a quantitative relation between the *global* effect of mutations on the *E. coli* proteome and bacterial fitness. We created *E. coli* strains with specific destabilizing mutations in the chromosomal *folA* gene encoding dihydrofolate reductase (DHFR) and quantified the ensuing changes in the abundances of 2,000+ *E. coli* proteins in mutant strains using tandem mass tags with subsequent LC-MS/MS. mRNA abundances in the same *E. coli* strains were also quantified. The proteomic effects of mutations in DHFR are quantitatively linked to phenotype: the SDs of the distributions of logarithms of relative (to WT) protein abundances anticorrelate with bacterial growth rates. Proteomes hierarchically cluster first by media conditions, and within each condition, by the severity of the perturbation to DHFR function. These results highlight the importance of a systems-level layer in the genotype-phenotype relationship.

INTRODUCTION

Understanding the genotype-phenotype relationship requires vantage points from multiple scales, ranging from the molecular, through the systems, to the cellular/organismal (Lehner, 2013). Several studies demonstrated that mutations in metabolic enzymes have local effects on fitness through changes in metabolic flux (Applebee et al., 2011; Dean et al., 1986; Soskine and Tawfik, 2010). Mutations that change protein stability can also affect fitness through modulation of the number of folded (active) proteins (Bershtein et al., 2006; Firnberg et al., 2014; Wylie and Shakhnovich, 2011) or by affecting the number of toxic unfolded species (Dobson, 2003; Drummond and Wilke, 2008).

However, in most cases, a direct link between the mutational effects on protein function and organismal phenotype is not obvious due to pleiotropic effects, such as protein aggregation (Drummond and Wilke, 2008) and formation of functional and non-functional multimers (Bershtein et al., 2012; Lynch, 2013; Zhang et al., 2008). Furthermore, recent studies have shown that partial inhibition of an enzyme can cause broad changes in the metabolic profile of the cell, extending far beyond the immediate products of enzymes in question (Kwon et al., 2008, 2010).

The systems-level proteomic response to a genetic variation is an important missing link in the multiscale genotype-phenotype relationship. Earlier studies showed that bulk characteristics of the macromolecular composition in the cell cytoplasm, e.g., the total protein concentration or the ratio of proteins to RNA, are sensitive to changes in growth conditions, such as the availability of nutrients (Ehrenberg et al., 2013; Klumpp et al., 2009). However, the effect of mutations or changed growth conditions on the abundances of individual proteins in the cytoplasm is not known. The key objective of the present study is to understand to what extent point mutations in a metabolic enzyme and/or variations in the media affect the proteome *composition* in the bacterial cytoplasm and how these changes are related to the fitness effects of such mutations.

We used isobaric tandem mass tag (TMT) proteome labeling with subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) to analyze changes in the *E. coli* proteome in response to a selected set of destabilizing mutations in the chromosomal copy of the *folA* gene (encoding the core metabolic enzyme dihydrofolate reductase [DHFR]) and found that these mutations reproducibly change the abundances of most detected *E. coli* proteins. Furthermore, we established that the proteome-level changes are directly related to the fitness effects of these mutations and/or media variation during the growth of the *E. coli* strains.

RESULTS

Effect of DHFR Mutations and Media Variations on *E. Coli* Fitness

folA is an optimal target for studying the genotype-phenotype relationship. First, its product is an important metabolic enzyme.