

Multi-omics data reveals the response of *Escherichia coli* to perturbations

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Abstract

Cellular systems are continuously exposed to various kinds of perturbations including extracellular environmental changes and/or intracellular genetic mutations. Functional robustness, which guarantees the maintenance of a stable phenotype in spite of perturbations, is often mentioned as a fundamental property of biological systems. Support for the existence of the biological robustness has been provided through some theoretical analyses or experimental studies with relatively small systems. However, there are few direct demonstrations of the existence of wide-range robustness in actual cellular systems. Here we show that robustness in living cells can be observed from comprehensive and quantitative analysis of intracellular components. We collected "multi-omics" data, defined here as comprehensive data sets about the main metabolic pathways obtained at various levels of biological information (1). Our data covers the transcriptome (quantitative RT-PCR and DNA microarray), proteome (quantitative LC-MS proteomics and 2D-DIGE), metabolome (CE-TOFMS (2)), and fluxome (GC-MS). Data was obtained for *Escherichia coli* BW25113 and single gene disruptants (Keio collection (3)) of 24 metabolic enzymes cultured in a chemostat at a dilution rate (DR) 0.2 h⁻¹. For the wild type strain, experiments using various DR were also performed. The data were normalized in a two-step process to reduce day-to-day variances in the raw data and to allow direct comparisons of variances among different types of the biochemical compounds. We refer to this normalized value as "Expression Index" (EI) and calculated an average expression index (AEI), for each type of monitored molecules (Figures 1, 2).

We then compared the AEs between samples. Games-Howell test was performed for all comparisons to test for statistically significant differences ($P < 0.05$).

In most cases, we observed an overall surprising stability in the AEs of transcripts/proteins/metabolites for most single gene disruptants. Two notable exceptions were *pfkA* or *rpiA* disruptants; in each mutant, the expression of an isozyme of the disrupted gene was strongly up-regulated due to suppressor mutations as previously reported (4, 5). In contrast, for most single gene disruptants, upon changes in specific growth rate, the AEs of metabolites were remarkably stable while significant changes were observed in AEs of transcripts and proteins. Same results were obtained from comparisons of AEs.

Our results suggest that *E. coli* can actively respond to changes in the concentration of the growth-limiting substrate by regulating the level of enzyme expression to maximize growth rate, which is reflected in the observed stability of metabolite levels. On the other hand, *E. coli* does not appear to respond significantly to the disruption of most single metabolic genes by regulating multiple other mRNA or protein levels. In this case, structural redundancy in the metabolic network itself likely provides the necessary robustness, allowing the levels of most metabolites to remain constant, although some localized perturbations are inevitable. Even if this strategy appears insufficient in the face of some mutations, *E. coli* may survive by accumulating additional mutations, as we observed for *pfkA* and *rpiA* disruptants. Using multiple strategies may thus enable *E. coli* to maintain a robust cellular phenotype when exposed to various types of perturbations.

References

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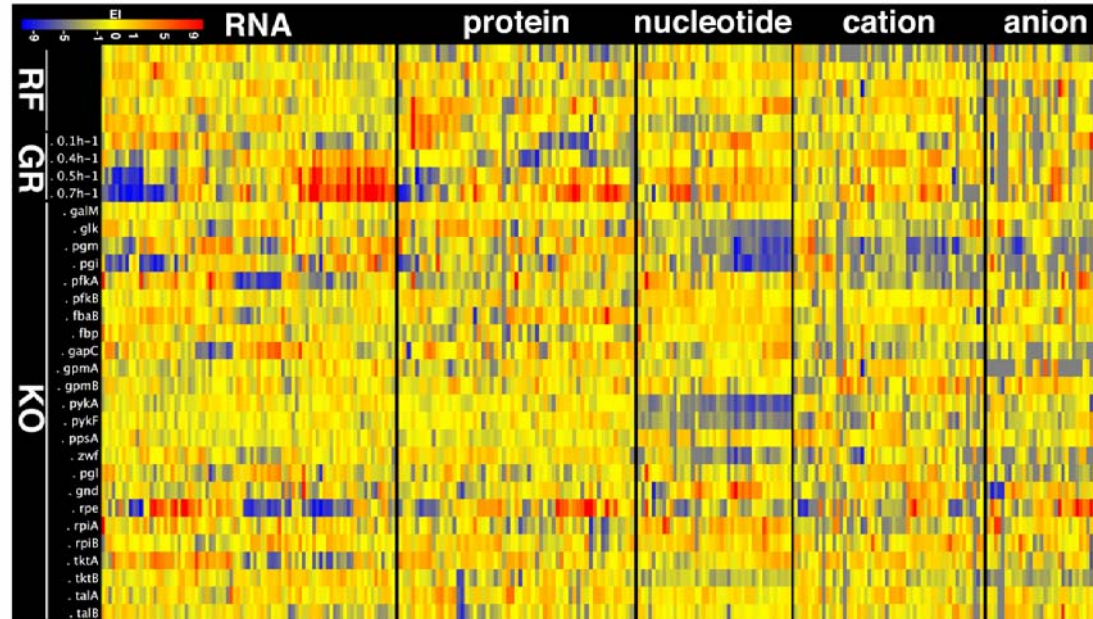


Figure 1: Overall representation of changes in the targeted cellular components. The heatmap shows the EI values of intracellular components that were detected in more than half the samples. RF, reference sample (wild-type cells cultured at a specific growth rate of 0.2 h^{-1}); GR, wild-type cells cultured at the indicated specific growth rates; KO, single gene knockout mutants cultured at a specific growth rate of 0.2 h^{-1} . The numbers of individual components shown are: metabolites, 130; proteins, 57; mRNA transcripts, 85.

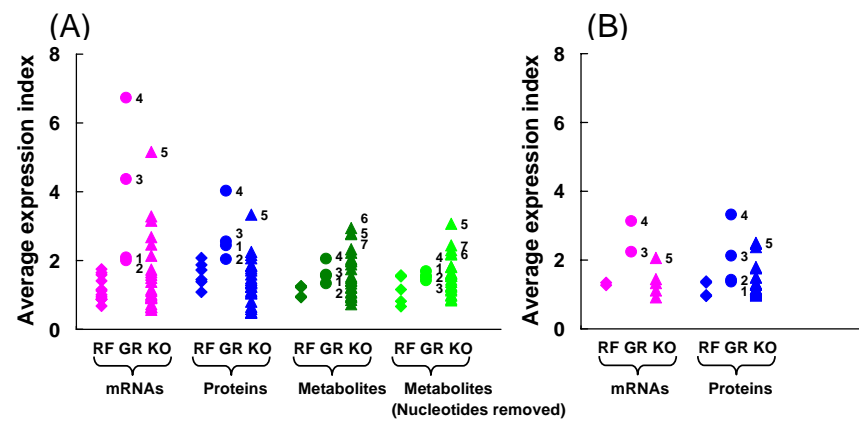


Figure 2: AEI values for mRNA, protein and metabolite levels. RF, GR and KO are defined in the legend of Figure 1. (A) Quantitative measurements obtained by targeted analysis (quantitative RT-PCR, quantitative LC-MS proteomics and CE-TOFMS metabolomics). (B) Semi-quantitative cell-wide measurements (DNA microarray and 2D-DIGE). In (A) and (B), numbers 1, 2, 3, and 4, correspond to specific growth rates of 0.1 , 0.4 , 0.5 , and 0.7 h^{-1} , and numbers 5, 6, and 7 correspond to *rpe*, *pgi*, and *pgm* disruptants, respectively.