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Genome-wide analysis of bacterial metabolic pathways

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Abstract

Systems analysis of the complete genome sequence combined with biochemical information on metabolic pathways has been used to define and elucidate the relationship between genotype and phenotype for *Haemophilus influenzae* Rd.

Significance and context

With the advent of complete genome sequence information comes the challenge of applying this information to physiological questions. At issue is the relationship between genotype and phenotype - both for a given organism in different environments, and for different organisms. Edwards and Palsson apply systems-based analysis to information derived from the annotated genomic sequence of the bacterium *Haemophilus influenzae* Rd and the biochemistry of its metabolic reactions to elucidate the metabolic physiology of *H. influenzae* Rd, an approach that can be applied to genotype-phenotype relationships in general. Having constructed an *in silico* metabolic genotype, they use it to ascertain critical metabolic components, to distinguish metabolic phenotypes for a given growth variable, and to determine essential, critical and redundant metabolic genes through the use of *in silico* gene deletion. This approach should advance our understanding of the underlying design principles of organisms, including the regulatory logic controlling metabolic pathways.

Key results

From biochemical and genomic information,the *H. influenzae* Rd metabolic genotype was defined as including 343 metabolites (m) and 488 metabolic reactions (n), and its characteristics were studied on the basis of the properties of its stoichiometric matrix (m x n). Flux-balance analysis using this matrix determined feasible solutions as the intersection of the solution sets satisfying mass-balance constraints and physicochemical constraints (for example, minimum and maximum flux values). Key metabolites of the *in silico H. influenzae* metabolic genotype were elucidated by examining the degree of connectivity of each of the metabolites, as determined by the number of metabolic reactions in which each of the 343 metabolites was used. Metabolites participating in the greatest number of reactions were deemed to be critical and included ATP, ADP, inorganic phosphate, pyrophosphate, carbon dioxide, glutamate, NADP, and NADPH, indicating that the generation of charged cofactors is critical. The capacity of a metabolic genotype to produce these cofactors was determined by optimizing their production in the

linear programming problem and comparing the charged cofactor capacities of *H. influenzae* and *E. coli* genotypes (see Figure 1).

In silico gene deletion experiments examined the effects of alterations in the metabolic genotype and the number of genes essential for *H. influenzae* Rd growth in defined media. Edwards and Palsson find that optimal use of the central metabolic pathways may be fundamentally different according to growth conditions, and that the number of redundant genes is greatly reduced when the analysis encompasses different growth conditions.

Figure 1 Phenotype phase diagram of the *H. influenzae* Rd metabolic phenotype (reprinted with permission from the *Journal of Biological Chemistry*).
The figure illustrates the six distinct metabolic phenotypes derived from the *H. influenzae* Rd *in silico* metabolic genotype when optimized for maximal biomass production under different conditions (mixed substrate with varied glutamate and fructose uptake rates). These metabolic phenotypes employ different combinations of pathways to optimize growth depending upon substrate availability, each with different constraining features that reflect the critical components (such as ATP) defined by connectivity analysis. In addition, they illustrate the complex relationship between pathway utilization and growth conditions.

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Links

LINDO 6.1, the linear programming software ia available from LINDO Systems, Inc (Chicago) and genomic information is organized into known biochemical pathways at the Kyoto encyclopedia of genes and genomes (KEGG), which also contains details of *Haemophilus. influenzae* Rd genes. Bernhard Palsson's current funding page contains further details of work carried out in his lab.

Reporter's comments

Use of this methodology requires complete, annotated genomic sequence and extensive biochemical information for the prokaryote in question. Tools such as KEGG (see above) are available to aid in defining the stoichiometric matrix. This paper beautifully couples phenotype to genotype and provides a mathematical framework for this coupling using flux-balance analysis that should be applicable not only for any prokaryote, but also for diverse phenotypes (that is, not just maximal growth). In addition, it shows the utility of *in silico* biology to elucidate complex relationships and to direct experimental work.

Table of links

Journal of Biological Chemistry

LINDO 6.1

Kyoto encyclopedia of genes and genomes

Haemophilus. influenzae Rd genes

Bernhard Palsson's current funding

Full text of *J Biol Chem* **274**:17410-17416

LINDO Systems, Inc

Access

The Full text of *J Biol Chem* **274**:17410-17416 is available to subscribers and non-subscribers online.

References

1. Edwards JS, Palsson BO: Systems properties of the *Haemophilus influenzae* Rd metabolic genotype. J Biol Chem. 1999, 274: 17410-17416. 0021-9293

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