Metabolic Flux Ratio Analysis of Genetic and Environmental Modulations of *Escherichia coli* Central Carbon Metabolism

UWE SAUER,¹* DANIEL R. LASKO,¹‡ JOCELYNE FIAUX,² MICHEL HOCHULI,² RALF GLASER,² THOMAS SZYPERSKI,²‡ KURT WÜTHRICH,² AND JAMES E. BAILEY ¹

Institut für Biotechnologie¹ and Institut für Molekularbiologie und Biophysik,² ETH Zürich, CH-8093 Zürich, Switzerland

Received 30 April 1999/Accepted 23 August 1999

The response of *Escherichia coli* central carbon metabolism to genetic and environmental manipulation has been studied by use of a recently developed methodology for metabolic flux ratio (METAFoR) analysis; this methodology can also directly reveal active metabolic pathways. Generation of fluxome data arrays by use of the METAFoR approach is based on two-dimensional $^{13}$C-$^1$H correlation nuclear magnetic resonance spectroscopy with fractionally labeled biomass and, in contrast to metabolic flux analysis, does not require measurements of extracellular substrate and metabolite concentrations. METAFoR analyses of *E. coli* strains that moderately overexpress phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase, or alcohol dehydrogenase revealed that only a few flux ratios change in concert with the overexpression of these enzymes. Disruption of both pyruvate kinase isoenzymes resulted in altered flux ratios for reactions connecting the phosphoenolpyruvate (PEP) and pyruvate pools but did not significantly alter central metabolism. These data indicate remarkable robustness and rigidity in central carbon metabolism in the presence of genetic variation. More significant physiological changes and flux ratio differences were seen in response to altered environmental conditions. For example, in ammonia-limited chemostat cultures, compared to glucose-limited chemostat cultures, a reduced fraction of PEP molecules was derived through at least one transketolase reaction, and there was a higher relative contribution of anaplerotic PEP carboxylation than of the tricarboxylic acid (TCA) cycle for oxaloacetate synthesis. These two parameters also showed significant variation between aerobic and anaerobic batch cultures. Finally, two reactions catalyzed by PEP carboxykinase and malic enzyme were identified by METAFoR analysis; these had previously been considered absent in *E. coli* cells grown in glucose-containing media. Backward flux from the TCA cycle to glycolysis, as indicated by significant activity of PEP carboxykinase, was found only in glucose-limited chemostat culture, demonstrating that control of this futile cycle activity is relaxed under severe glucose limitation.

Access to complete genome sequence information for a number of microorganisms now motivates the development and application of experimental techniques for phenotype characterization (such as transcriptome and proteome analyses), providing arrays of data that can be directly mapped to corresponding arrays of genes (14, 36). The physiological counterpart to such composition arrays is the array of fluxes (reaction rates on a per-unit cell volume or per-unit cell mass basis) for all of the reactions that occur in the organism, for which we use, by analogy, the term fluxome. Approximate fluxome access for certain subsets of metabolism can be attained by methods of metabolic flux analysis, which require data on uptake and efflux rates of certain metabolites outside the cell and which assume a corresponding network of metabolic pathways in the cell (39). Alternatively, by use of more recently introduced methodology based on isotopic imprinting of amino acids by their precursors, the active central carbon pathways and the ratios of their fluxes can be directly determined from two-dimensional (2D) nuclear magnetic resonance (NMR) analysis of hydrolyzed cell proteins (30–33). This method, for which we introduce the term METAFoR (metabolic flux ratio) analysis, offers a relatively high throughput access to these key fluxome elements, enabling physiological data arrays to be acquired over a broad range of genetic and environmental conditions.

Specifically, METAFoR analysis quantifies the relative abundance of intact carbon bonds originating from uniformly isotopically labeled source molecules by use of proton-detected 2D $^{13}$C-$^1$H correlation NMR spectroscopy (COSY) (30, 34, 42). Such 2D NMR analysis of amino acids obtained from hydrolyzed cell protein permits quantitative analysis of the relative abundance of intact, contiguous fragments in the precursor metabolites of central metabolism, because the carbon backbone of these molecules is conserved in the amino acids. Typically, fractional $^{13}$C labeling of amino acids is achieved by growing cells with a mixture of 85 to 90% natural-abundance glucose and 10 to 15% [U-$^{13}$C$_6$]glucose (22, 27, 30–32). Because alternative pathways leading to common intermediates or products produce different intact fragments originating from a single glucose source molecule (30–32), specific multiplet patterns in the $^{13}$C fine structures that reflect the in vivo usage of reactions are generated. Probabilistic equations relate the determined intensities of the multiplet components to the relative abundance of intact carbon fragments (30) and thus allow derivation of intracellular carbon flux ratios (30–33). These data provide not only comprehensive insight into cellular metabolism but also inherent flux indications that can provide critical information for metabolic (net) flux analysis (27, 32).

The active pathways and the flux distribution in central carbon metabolism are critical components of a multidimensional...