Classic Spotlight: Metabolic Flux—Which Way To Go?

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The Embden-Meyerhof-Parnas (EMP) pathway, more simply known as glycolysis, is typically the first pathway presented in biochemistry courses, where we learn that it is responsible for catabolism of glucose (Fig. 1). While this fundamental idea is certainly true, it overlooks the incredible metabolic diversity of the microbial world, where a variety of glucose catabolic pathways exist. Accordingly, many microorganisms catabolize glucose via the Entner-Doudoroff (ED) pathway, while others rely on the pentose-phosphate pathway (sometimes called the hexose-mono-phosphate shunt) (Fig. 1) (1). Significantly, even when the EMP pathway is the predominant mode of sugar catabolism, it is often dispensable, as illustrated by the ability of glycolytic mutants to grow on glucose (2).

In hindsight, the dispensability of glycolysis may seem obvious. We now know that biochemical pathways are often branched and that metabolic flux must be partitioned to balance the diverse catabolic and anabolic requirements of living cells. We also know that living organisms have remarkable biochemical plasticity, allowing them to reroute metabolic flux in the face of mutational or nutritional challenges. Indeed, characterization and modeling of the fluxome, defined as the complete set of metabolic fluxes in a cell, has become a hot topic in recent years, essential for the development of whole-cell models and rational metabolic engineering (3). Modern fluxomics is based largely on tracing of stable iso-

tope-labeled substrates with mass spectrometry or nuclear magnetic resonance (isotopomer analysis) and on systems-level modeling of flux balances (flux balance analysis [FBA] modeling) (4, 5). In the face of these powerful analytical methods, it is easy to forget how much can be learned from simple phenotypic analysis of mutants in model systems such as *Escherichia coli*.

A beautiful example of this early experimental approach is provided in an article by Fraenkel and Levisohn published in the *Journal of Bacteriology* in 1967 (6). This classic paper, reporting one of many related studies conducted in the Fraenkel lab, describes the isolation and characterization of an *E. coli* *pgi* mutant lacking phosphoglucose isomerase, which catalyzes the third step in the EMP pathway. The ability of this mutant to grow, albeit slowly, using glucose as the sole carbon and energy source clearly demonstrates the existence of alternate catabolic routes. Using a

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FIG 1 Abbreviated pathways for carbohydrate utilization in *Escherichia coli* (6). The Embden-Meyerhof-Parnas (EMP) pathway is shown in red, the Entner-Doudoroff (ED) pathway is in blue, and the pentose-phosphate pathway (hexose-monophosphate shunt) is in green.

technique that presaged modern isotopomer analysis, the researchers traced the fate of radiolabeled glucose, showing that the mutant metabolized glucose via the pentose-phosphate pathway and excluding the possibility that the Entner-Doudoroff pathway was involved. Through a series of biochemical assays, they also showed that the enzymes of the Entner-Doudoroff pathway were produced only during growth on gluconate, even when glycolysis was impaired. Perhaps the most significant finding in this paper was that the growth rate of the mutant on glucose was only one-third that of the parental strain, suggesting that metabolic flux through the pentose-phosphate pathway was significantly less than metabolic flux through glycolysis and that it was not increased when glycolysis was disrupted.

Today, we know that most organisms catabolize glucose via a combination of glycolysis and the pentose-phosphate pathway, with circa one-third flowing through the latter (note the similarity of this fraction to the mutant growth rate discussed above). Flux through the pentose-phosphate pathway provides the cell with a significant fraction of the NADPH required for biosynthesis, a finding that was demonstrated in a later publication by Csonka and Fraenkel (7). These and other studies provided an early win-

dow into the workings of the cell, showing that multiple catabolic pathways are available to particular substrates and that metabolic decisions regarding which way to go have a profound effect on the biology of living organisms.

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