The cost of efficiency in energy metabolism

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In a universe being dragged into disorder by the second law of thermodynamics, living cells must expend energy to maintain their complex organization. In addition to providing a carbon source for biosynthesis, the classical Embden–Meyerhof–Parnas (EMP) and Entner-Doudoroff (ED) pathways help to satisfy this energetic demand by generating ATP during glucose metabolism (1). Based on simple stoichiometry of reactants and products, the EMP pathway appears, at first blush, greatly preferable to the ED pathway, yielding twice as much ATP per glucose. If glucose breakdown and energy conservation are tightly coupled, why is the less-efficient ED pathway so prevalent? What has kept prokaryotic life in its entirety from casting off the ED pathway in favor of the more profitable EMP pathway? In PNAS, Flamholz et al. (2) address these questions by drawing on thermodynamics, enzyme kinetics, mathematical optimization, and genomics.

The first stage of glycolysis is characterized by an investment of ATP to phosphorylate glucose, which, so primed, is cleaved into two three-carbon intermediates (Fig. 1). The cell recoups its investment in the second phase of glycolysis (known as "lower glycolysis"), where oxidation of three-carbon intermediates directly generates ATP. Although the ED and EMP pathways overlap in part, they conspicuously differ in the number of three-carbon intermediates shunted down lower glycolysis. In the EMP pathway, glucose is phosphorylated twice, consuming two ATP, and both three-carbon intermediates (glyceraldehyde 3-phosphate, or G3P) enter lower glycolysis to produce two ATP each. In the ED pathway, glucose is only phosphorylated once, consuming one ATP, before being cleaved into one G3P and one pyruvate. The single G3P yields two ATP as in the EMP pathway, but pyruvate bypasses the bulk of lower glycolysis, foregoing ATP production. Thus, despite both pathways starting and ending with the same amount of glucose and lactate, the EMP pathway manages to extract two ATP per glucose, the ED pathway only one. The ED pathway is thought to predate the EMP pathway (dominant among eukaryotes) in the evolutionary timeline (3). Is the EMP pathway simply a fine-tuned adaptation of the ED pathway optimized for energy conservation, or does high ATP yield come at a cost?

Flamholz et al. (2) highlight a tradeoff that logically arises between a glycolytic pathway's ATP yield and thermodynamic driving force. Free energy released during glucose breakdown can drive ATP synthesis, providing energy currency to the cell, or dissipate as heat, making the overall pathway more thermodynamically favorable (albeit less efficient) (4). By harvesting more free energy as ATP than the ED pathway, the EMP pathway operates closer to equilibrium (5). Conversely, production of pyruvate so early in the ED pathway–a highly exergonic reaction–dissipates ample free energy, leaving little for ATP generation. Flamholz et al. (2) show that, even if metabolites assume concentrations that make the least favorable reactions in each pathway as exergonic as possible, the EMP pathway faces much tighter thermodynamic bottlenecks than the ED pathway. Although a simplified representation of linear metabolic pathways (as in Fig. 1) conveys their tendency to proceed in the forward direction, it glosses over bottleneck reactions that are only weakly favorable, with a negative free-energy difference close to zero. Such highly reversible reactions can easily clog a pathway, limiting if not preventing forward flux.

Despite its thermodynamic obstacles, the EMP pathway is known to function effectively in living cells. How to reconcile these two contrasting observations? And how would we expect a microorganism to cope physiologically with a thermodynamic bottleneck? Flamholz et al. (2) address these questions by considering the enzyme production costs associated with each pathway.

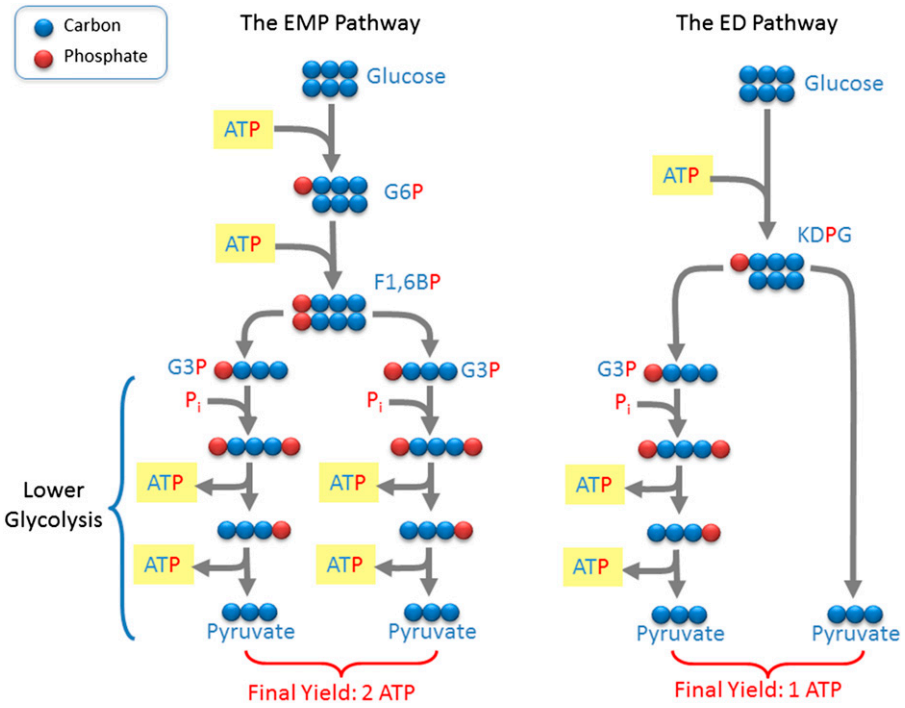

Fig. 1. Structural differences between the ED and EMP pathways. This simplified diagram focuses on a few key aspects of the ED and EMP pathways (i.e., the flow of carbon and phosphate groups) to highlight the different organization of ATP-consuming and ATP-producing steps, leading to different ATP yields. Other important details (such as the stoichiometry of reducing equivalents NADH and NADPH) can be found in traditional depictions of these pathways. G6P, glucose 6-phosphate; F1,6BP, fructose 1,6-bisphosphate; G3P, glyceraldehyde 3-phosphate; Pi, inorganic phosphate.

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In its traditional form, the Michaelis–Menten equation predicts the rate of a reversible reaction as a function of kinetic parameters and the concentrations of enzyme and metabolites. Flamholz et al. cleverly use the Haldane relationship to rewrite the Michaelis–Menten rate law, expressing the reaction rate as a product of the enzyme level, a kinetic term that quantifies distance from substrate saturation, and a thermodynamic term (called the Net Flux Ratio) that quantifies distance from equilibrium. This expression shows how a high enzyme level can kinetically compensate for a reaction's close proximity to equilibrium. One can now calculate, for each reaction, the amount of enzyme necessary to bring about a given overall flux (or steady-state reaction rate) through the pathway, in light of thermodynamic and kinetic parameters. More precisely, Flamholz et al. (2) use constraint-based optimization to find the metabolite and enzyme concentrations that minimize a pathway's protein cost, defined as the product of enzyme abundance and molecular mass summed over all reactions. This approach falls in line with early optimization methods developed by Ebenhöh and Heinrich (6) and recent constraint-based models of metabolism, where assumed cell-level objectives partially compensate for missing knowledge (7).

Flamholz et al. (2) find that the high-yield EMP pathway incurs a greater protein cost (3.5-fold) than the ED pathway, necessitating higher enzyme levels to support the same flux. In effect, enzyme levels must strategically increase in the EMP pathway to counterbalance low thermodynamic driving force. According to experimental measurements cited by Flamholz et al., enzymes catalyzing the EMP pathway make up a sizable proportion of the proteome in both *Escherichia coli* and *Saccharomyces cerevisiae*. Altogether, Flamholz et al. depict a thermodynamically constrained EMP pathway driven forward by costly enzymatic machinery in the interest of high ATP yield. The ED pathway, by virtue of its low ATP yield, emerges as thermodynamically relaxed, requiring lower enzyme levels to operate. Interestingly, a previous computational analysis of the metabolic network in *E. coli* (based on elementary flux modes) also characterized the ED and EMP pathways as designed to reduce "investment cost" (high protein synthesis) and "operating cost" (poor ATP yield), respectively (8).

What circumstances in nature tip the scale between maximizing ATP yield and minimizing protein synthesis? Flamholz et al. (2) suggest that prokaryotes will adopt one strategy or the other depending on whether substrate-level phosphorylation makes up a small or large fraction of total ATP production. In completing the breakdown of lactate to CO_2 downstream from glycolysis, aerobes produce 25–30 ATP through oxidative phosphorylation, rendering a single extra ATP per glucose dispensable. For anaerobes, which rely on weaker oxidants than O_2 for respiration or use fermentation, substrate-level phosphorylation represents the primary means of ATP production, justifying a greater investment in enzyme synthesis. By identifying enzymes unique to each pathway [e.g., 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase in the ED pathway (9)] in over 500 annotated genomes, Flamholz et al. (2) study the distribution of glycolytic pathways among heterotrophic prokaryotes, and find that, as expected, anaerobes overwhelmingly favor the EMP pathway, while the ED pathway is statistically overrepresented among aerobes.

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It is worth noting the relationship between the tradeoff studied by Flamholz et al. (2) and previous analyses of tradeoffs between metabolic rate and metabolic yield (5). In their exploration of ATP yield vs. protein cost, Flamholz et al. fix the flux through two pathways with different ATP yields and weigh the resulting protein costs. One could alternatively consider the same system under a fixed protein cost, in which case high yield comes at the expense of high flux (or reaction rate) (5). More broadly, the study of tradeoffs between the metabolic benefits of sustaining flux along a certain pathway, and the corresponding enzyme production cost, is emerging as a valuable gateway to several open research questions, from predicting evolutionary dynamics and epistasis (10, 11) to understanding the logic of metabolic regulation in the cell (12). In parallel, there is renewed interest in thermodynamic aspects of metabolism, with efforts to include nonequilibrium thermodynamics and energy balance constraints alongside flux balance constraints in stoichiometric models of metabolic networks (13–15). The work by Flamholz et al. (2) offers an exciting perspective at the crossroads of these trends.

Faced with the dizzying output of almost 4 billion years of evolution, many biologists strive for a comprehensive understanding of the underlying principles that shaped cellular metabolism as we know it today. Flamholz et al. (2) dive in particular into the biological rationale for widespread adoption of the seemingly inefficient ED pathway. The unique insights they offer into this classical pathway (discovered in *Pseudomonas saccharophila* in 1954) (16) are enabled by recent advances in systems biology, such as the high-throughput sequencing and annotation of genomes and the rise of computational tools like constraint-based optimization. In future efforts, genome-scale metabolic models (7) might shed light on whether production of NADPH in the ED pathway, another key distinction with the EMP pathway, has a significant impact on the overall metabolic budget of the cell. Further experimental validation of the broader capacity to infer enzyme levels based on thermodynamics and kinetics would have strong implications in fields as diverse as metabolic engineering and evolutionary biology.

1 White D (2006) *The Physiology and Biochemistry of Prokaryotes* (Oxford Univ Press, Oxford).

2 Flamholz A, Noor E, Bar-Even A, Liebermeister W, Milo R (2013) Glycolytic strategy as a tradeoff between energy yield and protein cost. *Proc Natl Acad Sci USA*, 10.107/pnas.1215283110.

3 Romano AH, Conway T (1996) Evolution of carbohydrate metabolic pathways. *Res Microbiol* 147(6–7):448–455.

4 Westerhoff HV, Hellingwerf KJ, Van Dam K (1983) Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate. *Proc Natl Acad Sci USA* 80(1):305–309.

5 Pfeiffer T, Schuster S, Bonhoeffer S (2001) Cooperation and competition in the evolution of ATP-producing pathways. *Science* 292(5516):504–507.

6 Ebenhöh O, Heinrich R (2001) Evolutionary optimization of metabolic pathways. Theoretical reconstruction of the stoichiometry of ATP and NADH producing systems. *Bull Math Biol* 63(1):21–55.

7 McCloskey D, Palsson BØ, Feist AM (2013) Basic and applied uses of genome-scale metabolic network reconstructions of *Escherichia coli*. *Mol Syst Biol* 9:661.

8 Carlson R, Srienc F (2004) Fundamental *Escherichia coli* biochemical pathways for biomass and energy production: creation of overall flux states. *Biotechnol Bioeng* 86(2):149–162.

9 Patra T, Koley H, Ramamurthy T, Ghose AC, Nandy RK (2012) The Entner-Doudoroff pathway is obligatory for gluconate utilization and contributes to the pathogenicity of *Vibrio cholerae*. *J Bacteriol* 194(13):3377–3385.

10 Chou HH, Chiu HC, Delaney NF, Segrè D, Marx CJ (2011) Diminishing returns epistasis among beneficial mutations decelerates adaptation. *Science* 332(6034):1190–1192.

11 Chiu H-C, Marx CJ, Segrè D (2012) Epistasis from functional dependence of fitness on underlying traits. *Proc Biol Sci* 279(1745): 4156–4164.

12 Reznik E, Yohe S, Segrè D (2013) Invariance and optimality in the regulation of an enzyme. *Biol Direct* 8(1):7.

13 Beard DA, Liang SD, Qian H (2002) Energy balance for analysis of complex metabolic networks. *Biophys J* 83(1):79–86.

14 Henry CS, Jankowski MD, Broadbelt LJ, Hatzimanikatis V (2006) Genome-scale thermodynamic analysis of *Escherichia coli* metabolism. *Biophys J* 90(4):1453–1461.

15 Fleming RMT, Maes CM, Saunders MA, Ye Y, Palsson BØ (2012) A variational principle for computing nonequilibrium fluxes and potentials in genome-scale biochemical networks. *J Theor Biol* 292:71–77.

16 Conway T (1992) The Entner-Doudoroff pathway: History, physiology and molecular biology. *FEMS Microbiol Rev* 9(1):1–27.