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Posttranslational regulation of microbial metabolism

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Fluxes in microbial metabolism are controlled by various regulatory layers that alter abundance or activity of metabolic enzymes. Recent studies suggest a division of labor between these layers: transcriptional regulation mostly controls the allocation of protein resources, passive flux regulation by enzyme saturation and thermodynamics allows rapid responses at the expense of higher protein cost, and posttranslational regulation is utilized by cells to directly take control of metabolic decisions. We present recent advances in elucidating the role of these regulatory layers, focusing on posttranslational modifications and allosteric interactions. As the systematic mapping of posttranslational regulatory events has now become possible, the next challenge is to identify those regulatory events that are functionally relevant under a given condition.

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Introduction

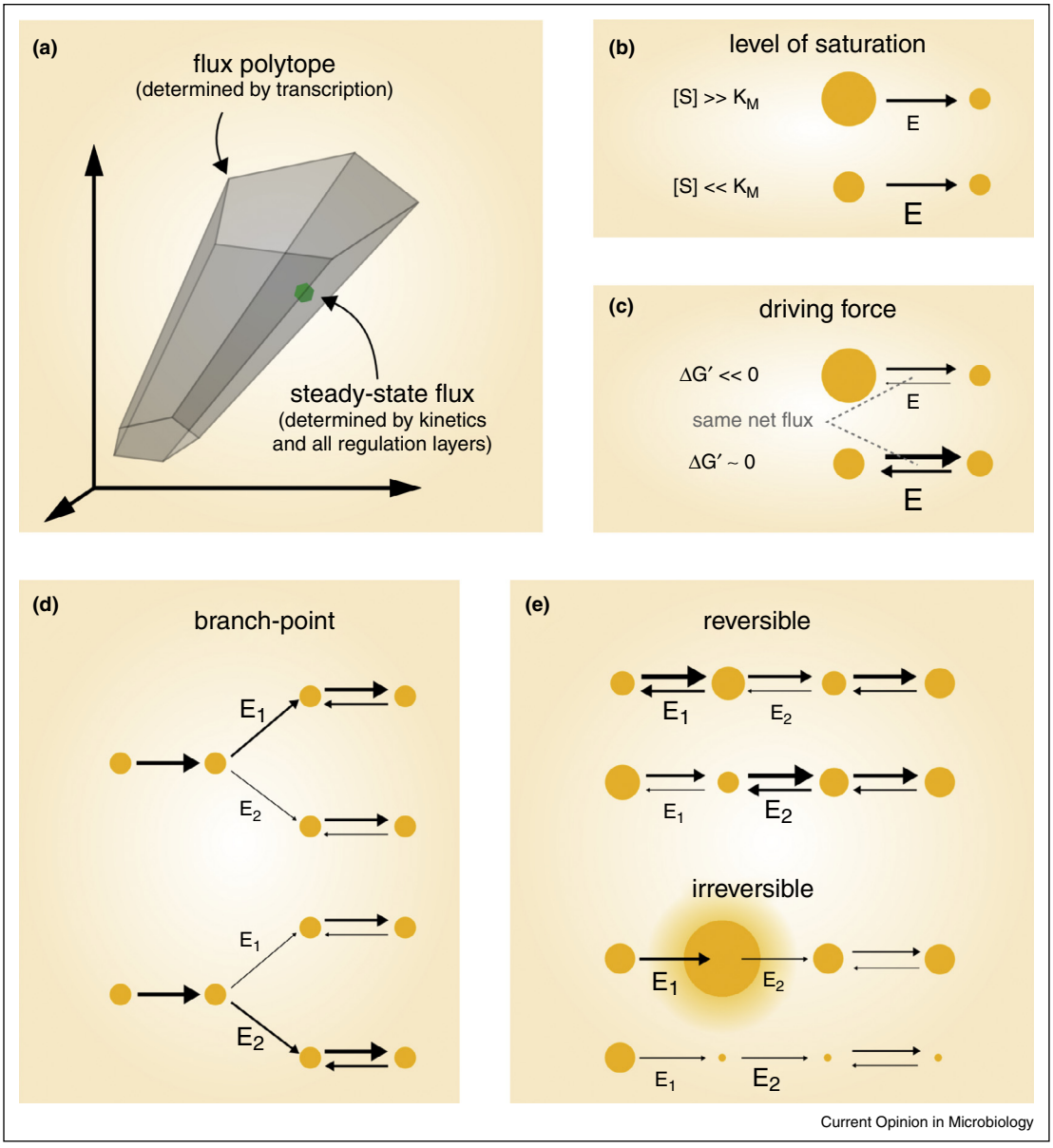
Regulation of metabolic fluxes lies at the core of many microbial processes and comprises a plethora of regulatory layers, such as transcriptional regulation, posttranslational modification, and allostery. All of these regulatory layers ultimately act by altering the capacity of metabolic enzymes through changes in their expression or activity. Arguably, the role of transcriptional regulation is better understood, and its importance for adapting to changes in nutrient availability and coordinating catabolism and anabolism is well established [1]. On the other hand, focusing on flux control in central metabolism paints a different picture, with so far little correspondence between flux and transcriptional changes [2,3]. Moreover, recent studies have demonstrated that transcriptional regulation can be surprisingly promiscuous and often driven by global physiological parameters such as the growth rate rather than the exact nature of the perturbation [4–7]. Also, the stochastic nature of transcription and translation events makes it difficult to precisely tune enzymes to their flux requirement [8–11]. These findings suggest that cells do not — and arguably cannot — use transcriptional regulation to fine-tune the expression of each metabolic enzyme in accordance to changes in flux. Instead, cells use transcriptional regulation to merely set the scope of possible fluxes (Figure 1a), and apply other regulatory layers to determine the exact location inside this space.

Recent reviews have discussed the prevalence of posttranslational modifications (PTMs) in microbial metabolic enzymes [12]. In this review, we highlight recent advances in elucidating how microbial metabolism is shaped by posttranslational regulation by PTMs as well as allostery. We start by outlining the mechanisms which enable cells to change metabolic fluxes even in the absence of designated regulation, and close by discussing the implications for the engineering of metabolic pathways.

When is posttranslational regulation really necessary?

Cells of highly organized multicellular organisms can cooperatively control their immediate extracellular environment to ensure homeostasis. Without that privilege, microorganisms must always be ready to respond quickly to unpredictably changing conditions — often by adjusting their internal fluxes accordingly. But, do they necessarily have to actively regulate all the enzymes whose flux is changing? Even in the absence of allosteric effectors or PTMs, the physical nature of enzyme kinetics allows flux to be regulated passively by changing the saturation level (substrate concentration relative to K_M value) or the thermodynamic driving force (for reversible reactions). For example, the flux through an unsaturated enzyme can be adjusted by altering the concentration of its substrate. Conversely, the flux through a reversible enzyme operating close to thermodynamic equilibrium can be changed or even reversed by moderately changing the ratio of its substrates and products (Figure 1b,c). Thus, enzyme kinetics and thermodynamics provide metabolic networks with considerable inherent flexibility to respond to environmental changes, and there is growing evidence that a large fraction of central metabolic enzymes may rely on these passive regulatory mechanisms. For example, quantitative metabolomics in *E. coli* revealed that the concentrations of many metabolites in central metabolism are around their respective K_M value [13]. Consequently,

Figure 1



When is regulation actually needed for controlling flux? **(a)** The stoichiometric space of feasible flux states is defined by the set of expressed enzymes, but the condition-specific fluxes are largely determined by enzyme kinetics and all layers of regulation including post-translational ones. **(b)** When an enzyme is not saturated, the flux will be sensitive to the concentration of substrate, but higher levels of the enzyme will be needed to achieve the same flux as in the saturated case. **(c)** Similarly, for the same net flux, a reaction closer to thermodynamic equilibrium ($\Delta G' \sim 0$) will be more sensitive to changes in substrate and product levels, but will require relatively higher amounts of the enzyme due to the counter-productive backward flux. **(d)** Branch-points are particularly sensitive to changes in enzyme levels, especially if a precise flux-ratio is required. **(e)** In a pathway comprised of reversible reactions the metabolite concentrations can partially compensate for the stochasticity in enzyme expression levels. If some reactions are irreversible, however, a significant imbalance between consecutive reactions can cause a deleterious accumulation of the intermediate compound, or a severe depletion that would slow down all downstream reactions.

these enzymes operate at saturation levels at which they are sensitive to changes in substrate concentrations. Further studies have also shown that many reactions in the central metabolism of *E. coli* and *S. cerevisiae* operate close to equilibrium, in particular in lower glycolysis [13–15,16•]. The enzymes catalyzing these reactions essentially operate in fire-and-forget mode, in which no additional regulation is needed for rapid metabolic changes. However, this mode of operation does come at a high cost: for enzymes operating close to equilibrium, the majority of their capacity is wasted on exchange fluxes without contributing to the net flux.