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Current Opinion in Microbiology

Metabolic-flux dependent regulation of microbial physiology

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According to the most prevalent notion, changes in cellular physiology primarily occur in response to altered environmental conditions. Yet, recent studies have shown that changes in metabolic fluxes can also trigger phenotypic changes even when environmental conditions are unchanged. This suggests that cells have mechanisms in place to assess the magnitude of metabolic fluxes, that is, the rate of metabolic reactions, and use this information to regulate their physiology. In this review, we describe recent evidence for metabolic flux-sensing and flux-dependent regulation. Furthermore, we discuss how such sensing and regulation can be mechanistically achieved and present a set of new candidates for flux-signaling metabolites. Similar to metabolic-flux sensing, we argue that cells can also sense protein translation flux. Finally, we elaborate on the advantages that flux-based regulation can confer to cells.

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Current Opinion in Microbiology 2017, **42**:71–78

This review comes from a themed issue on **Cell regulation**

Edited by **Jan-Willem Veening** and **Rita Tamayo**

https://doi.org/10.1016/j.mib.2017.10.029

Introduction

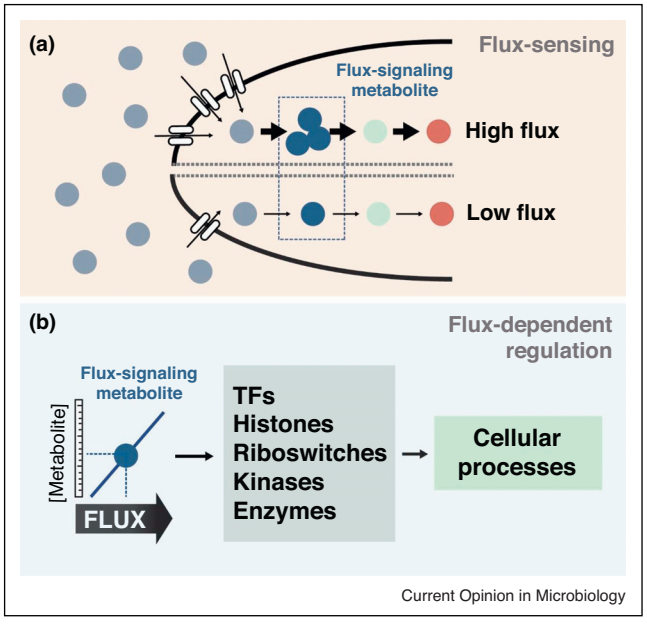
Microorganisms are often confronted with changes in their environment, for instance, in terms of nutrient availability. Direct assessment of the extracellular conditions, for example through two-component systems in bacteria [1], often leads to adaptations in response to environmental changes. However, there is increasing evidence showing that microbial cells can display changes in their phenotype, for example, in growth rate, gene expression and metabolism, also in response to changes in intracellular metabolic fluxes, even when the extracellular conditions are kept constant [2–5]. But does this flux-dependent regulation have a major impact on cell physiology? How can cells mechanistically sense metabolic fluxes, that is, rates of enzymatic reactions and metabolic pathways, and use this information for regulation? And why is this regulation advantageous to the cell?

Microorganisms display flux-dependent phenotypes

Accumulating evidence suggests that microbial cells can display phenotypes imposed by metabolic fluxes, and not directly by extracellular conditions. One example is the switch from respiratory to fermentative metabolism in glucose-rich conditions. When both *Escherichia coli* and yeast were grown in the same nutrient environment, but the rate of sugar uptake was controlled by inducible expression of sugar permeases or by using hexose transporter variants with different kinetics (Figure 1a) respectively, a glycolytic flux-dependence of the metabolic mode — a respiratory or fermentative metabolism — was found [4,6]. A meta-analysis of data from a number of studies that used different yeast strains grown under different conditions suggested that this switch is triggered when a specific sugar uptake rate is exceeded [7]. Because the onset of ethanol production is accompanied by a decrease in the oxygen uptake rate, this study suggested that this 'overflow metabolism' is an active response to the level of glycolytic flux, rather than a limitation in oxidative metabolism.

Intracellular flux changes under constant environmental conditions can also re-shape proteome expression. Proteome analyses carried out on bacteria grown in lactose as the sole carbon source but in which metabolic fluxes were modulated by titrating the expression of either the lactose permease or the enzyme involved in ammonia assimilation showed that as much as 50% of the proteome was altered in these conditions [8•]. Similarly, a comprehensive fluxomics and proteomics analysis in *S. cerevisiae* strains which were grown in the same environment but had different hexose uptake capacities, found that the expression of nearly half of ≈200 quantified metabolic proteins changed in a flux-dependent manner. Proteins whose expression correlated positively with glycolytic flux were found to be enriched for glycolytic proteins. On the other hand, proteins with expression levels negatively correlating with glycolytic flux were enriched for proteins involved in the TCA cycle, and in pyruvate, glyoxylate and dicarboxylate metabolism [3].

Figure 1



Schematic illustration of flux-sensing and flux-dependent regulation. **(a)** Kinetics of enzymes and their regulation is such that certain intermediates become flux-signaling metabolites, that is, their levels either correlate (or anticorrelate) with metabolic flux. At constant nutrient conditions, differences in metabolic fluxes can be achieved through variations in nutrient transporter levels (illustrated in the scheme), or through variations of flux-limiting enzymes. **(b)** Information about metabolic flux is imprinted into the concentration of a flux-signaling metabolite, which then interacts with regulatory factors and enzymes (grey box) to control other cellular processes (including metabolism). *Abbreviation*: TFs, transcription factors.

Apart from determining the metabolic mode and dictating protein expression, metabolic fluxes control growth. Glucose influx determines growth rate in *S. cerevisiae* [2,4], and in *E. coli* [9]. In addition, the fraction of *E. coli* cells that enters persistence, a state of no or slow-growth characterized by antibiotic tolerance, was shown to anticorrelate with glucose influx when the ratio between glucose and a non-metabolizable analogue was modulated [10]. Similarly, after a nutrient shift of *E. coli* from glucose to fumarate, persister cells are formed, and the rate of persister formation correlates negatively with fumarate uptake rate [11,12•]. While the most prevalent notion has been that persistence is triggered by toxin–antitoxin systems, recent work demonstrated that previous findings considering toxin–antitoxin systems contained artifacts [13]. Thus, as suggested by the above-mentioned findings, persistence entry is likely metabolic flux-dependent.

How do cells measure and use fluxes for regulation?

The important question that arises is how cells are capable of assessing the level of metabolic flux, and use this information for regulation. Changes in flux, induced by environmental changes or stochastic expression of transporters or enzymes, could be assessed by changes in the concentration of pathway intermediates (Figure 1a). However, the concentrations of metabolites are determined by the combination of the kinetics of the consuming and producing reactions. Metabolite concentrations do not necessarily change when fluxes are altered [14•], nor do they necessarily scale with flux [15]. Therefore, to accomplish flux-sensing via the concentration of certain metabolites, specific kinetics of the involved enzymes and specific regulation of these enzymes are required, such that the strict correlation (or alternatively, anti-correlation) between the metabolite concentration and metabolic flux is an emerging behavior. We refer to metabolites with such a behavior as *flux-signaling metabolites*.

The glycolytic intermediate fructose-1,6-bisphosphate (FBP) has been identified as a flux-signaling metabolite [16•]. FBP levels correlate with glycolytic flux across a broad range of microbial species and conditions [3,7,14•,17•,18–20], and even in dynamic perturbations of glycolysis [21]. It has been found recently that the molecular system translating the glycolytic flux into the FBP level encompasses all enzymes of lower glycolysis including the feedforward activation of pyruvate kinase by FBP, which ensures that FBP concentration correlates linearly with glycolytic flux over a broad range of fluxes [17•].

To transduce the flux information 'stored' in the concentration of a flux-signaling metabolite (e.g. FBP) into a response, a concentration-dependent interaction between the flux-signaling metabolite and other cellular components is required. In fact, it is well documented that metabolites interact with and regulate metabolic enzymes [22], transcription factors [23,24], protein kinases [25,26], and *cis*-regulatory RNA sequences (*riboswitches*) (Figure 1b). Additionally, some metabolites (e.g. acetyl-CoA) can have a critical role in the expression of specific genes because they are utilized as substrate for covalent modifications of histones [27,28]. However, the physiological relevance of such interactions in most cases is still unclear. Most available information stems from *in vitro* studies focusing on purified individual proteins or RNA species [29], in part because direct perturbation of metabolite levels in living cells without off-target effects is still impossible. However, by systematically investigating metabolites that affect transcriptional regulation *in vivo*, Kochanowski and co-workers showed that indeed (flux-signaling) metabolites (cyclic AMP, FBP, and fructose-1-phosphate) interacting with two major transcription factors (Crp and Cra) are responsible for the majority of the transcriptional regulation observed across 23 diverse growth conditions in *E. coli* [30].

While previously interactions between metabolites and other cellular molecules were mostly found by