Chapter 2

A flux-sensing mechanism could regulate the switch between respiration and fermentation

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Abstract

The yeast Saccharomyces cerevisiae can show different metabolic phenotypes (e.g. fermentation and respiration). Based on data from the literature, we argue that the substrate uptake rate is the core variable in the system that controls the global metabolic phenotype. Consequently the metabolic phenotype that the cell expresses is not dependent on the type of the sugar or its concentration, but only on the rate at which the sugar enters the cell. As this requires the cells to ‘measure’ metabolic flux, we discuss the existing clues toward a flux-sensing mechanism in this organism and also outline several aspects of the involved flux-dependent regulation system. It becomes clear that the sensing and regulation system that divides the taken up carbon flux into the respiratory or fermentative pathways is complex with many molecular components interacting on multiple levels. To obtain a true understanding about how the global metabolic phenotype of S. cerevisiae is controlled by the glucose uptake rate, different tools and approaches from systems biology will be required.
Introduction

Microorganisms are constantly facing changing environments, for example in terms of their nutrient availability. To be informed about the characteristics of the environment, sensory systems are required. Classically, these are either trans-membrane receptors (1, 2) or intracellular receptors (such as transcription factors) whose activity is modulated by their specific ligands. While for certain substrate molecules, we know such sensory systems [in yeast, e.g. the trans-membrane receptors Snf3p and Rgt2p (3)], or the intracellular receptor Gal3p (4, 5), for many other substrates, we have not yet identified specific sensors. The question is whether we simply do not know them yet or whether cells recognize these metabolites in a different way.

An alternative way to sense the presence of a certain carbon source would be by measuring the metabolic flux that is derived from its degradation. In this way, cells could perceive what substrate is being imported and at what rate. However, currently known sensing mechanisms in biology are based on concentration measurements. Yet, it has been shown for *Escherichia coli* in a synthetic, engineered system that it is possible for cells to ‘measure metabolic fluxes’ and use these for regulation (6). In addition, a recent computational study that modeled *E. coli* central metabolism suggested that intracellular metabolic fluxes can indeed be used to indirectly perceive the presence of a particular carbon source and the rate at which it enters the cell (7). Also for yeast, it was occasionally proposed that it can measure metabolic flux (8-10), but it was similarly often explicitly stated that this would not be possible (11-13).

In this perspective article, we explore flux-sensing and flux-dependent regulation in yeast. Specifically, we look at: (i) what kind of evidence or indication we have for flux-dependent regulation, (ii) general conceptual issues associated with sensing a rate and at potential flux-sensing mechanisms in yeast and (iii) some of the properties of the regulatory machinery that controls metabolism in a flux-dependent manner. As flux-sensing and flux-dependent regulation will not be accomplished via a single molecule, but rather will be realized by a whole system of molecules interacting in a very specific and likely complicated manner, systems biology approaches and modeling will definitely be required to generate a comprehensive understanding about this system. We conclude with a brief discussion about how systems biology could be instrumental toward this goal.

Evidence for metabolic flux-dependent regulation

In the literature, it is often suggested, explicitly or implicitly, that there is a relationship between glucose uptake rate and the usage of the respiratory and fermentative pathways, e.g. (8, 14-16). As a correlation between a certain metabolic flux and a particular phenotype would be a good indication for flux-dependent regulation, we set out to investigate how respiration, fermentation, and glucose uptake rate are linked. As a marker for the amount of respiration or fermentation, we used ethanol production rates and plotted these against the glucose uptake rate. As a first step, data obtained from various aerobic glucose-
limited chemostat cultures of *Saccharomyces cerevisiae* (17-24) showed indeed a good correlation between glucose uptake rate and ethanol production rates [cf. Inset of Figure 1a].

In contrast to limiting the glucose uptake rate through environmental conditions, manipulating the maximal glucose uptake rate by genetic means is an alternative approach to assess the importance of the glucose uptake rate in dictating the metabolic mode. Elbing *et al.* (14) constructed a set of hexose transporter mutants that only differ in their maximum glucose uptake rate. Similar to the results obtained for the various glucose-limited chemostat cultures, it was found that dependent on the rate of glucose uptake, the different transporter strains metabolize glucose to various extents by respiration and fermentation in an identical (*i.e.* high glucose) environment (14). These data perfectly align with the above-mentioned chemostat data (cf. inset of Figure 1a). For example, when grown in a glucose batch culture, the phenotype of the strain with the lowest glucose uptake capability (TM6*) is the same as that of the wildtype in a chemostat culture at a low dilution rate when the glucose influx is restricted by

**Figure 1.** (a) Ethanol production rate as a function of sugar uptake rate (normalized to c-mol); inset: wild-type *Saccharomyces cerevisiae* on glucose batch cultures (open squares); wild-type *S. cerevisiae* on glucose-limited chemostat cultures (open triangles); *S. cerevisiae* hexose transporter mutant strains on glucose batch cultures (open diamonds), arrows indicate measurement values of the TM6* strain (see main text); *S. cerevisiae* glucose sensing mutant strains on glucose chemostat cultures (circles); main: *S. cerevisiae* data from inset pooled (squares); environmentally and genetically perturbed *S. cerevisiae* on glucose, incl. anaerobic conditions (triangles); *S. cerevisiae* wildtype and mutants on other sugars with different environmental conditions (circles); other wild-type yeast species on glucose (diamonds). (b) Ethanol production rate as a function of the growth rate; *S. cerevisiae* data from inset pooled (squares); environmentally and genetically perturbed *S. cerevisiae* on glucose, incl. anaerobic conditions (triangles); *S. cerevisiae* wildtype and mutants on other sugars with different environmental conditions (circles); other wild-type yeast species on glucose (diamonds). In some cases, data were not available for both the ethanol production rate and the growth rate.
environmental conditions. As a result the glucose uptake rate seems to be the control factor for the metabolic mode and not environmental conditions such as, for example, the extracellular glucose concentration. Indeed, also glucose sensing mutants (18) switch from respiration to fermentation at similar glucose uptake rates to the corresponding wild-type strain (cf. Inset of Figure 1a). Also data from various different S. cerevisiae strains grown in glucose batch cultures (14, 25-35) fit nicely to the so far presented data [cf. Inset of Figure 1a].

As the correlation between glucose uptake rate and ethanol secretion rate seems to be independent of the culturing methods (e.g. chemostat, batch cultures), the glucose concentration, and the S. cerevisiae strains used, we asked whether glucose uptake rates and ethanol production rates were still linked in a similar fashion under anaerobic conditions. As can be seen from Figure 1a, data from anaerobic cultures (24, 33, 36-38) fit well with the correlation presented in the inset of Figure 1a. Along the same lines, data from yeast grown under various environmental conditions, such as different pH or salinity of the medium (30), temperature (30, 39, 40), addition of weak organic acids (21, 41, 42), and nitrogen-limited chemostats (19, 42, 43), also align well with the already plotted data (cf. Figure 1a). Even when data from a wide range of S. cerevisiae mutant strains are added, the correlation between glucose uptake rate and ethanol secretion rate still remains intact. Examples of such mutant strains include strains with overexpressed enzymes of lower glycolysis (33), uracil auxotrophies (17), single deletions or overexpression of various proteins involved in glucose regulation (26, 34) and carbon metabolism (25, 28, 35).

Because of this robustness of the correlation between the glucose uptake and ethanol secretion rates, we next asked whether this correlation would also be maintained in different yeast species. Yeast species can roughly be subdivided in Crabtree positive and Crabtree negative yeast (44). Although the term ‘Crabtree effect’ has been used with different meanings (45, 46), here ‘Crabtree positive’ refers to the ability of a certain yeast species to produce ethanol under aerobic conditions (44, 47). It is sometimes argued that Crabtree negative yeasts have a higher respiratory activity than Crabtree positive yeasts and, as a result, Crabtree negative yeasts do not have to resort to ethanol formation under high glucose conditions (48). However, data from various Crabtree positive and negative yeast species (27, 49) showed in general a similar relationship between glucose uptake and ethanol production as S. cerevisiae. This suggests that certain Crabtree negative yeast species have simply a lower glucose import rate and thus show no fermentative activity; in line with what was also suggested earlier (49-52). This idea is further supported by the observation that Crabtree negative yeast species (Candida utilis, Hansenula polymorpha, and Kluyveromyces marxianus) can ferment under aerobic conditions by stimulating glucose uptake using a weak organic acid (47) (data not shown).

Generally, S. cerevisiae ferments glucose, but other sugars, such as sucrose, mannose and galactose, are respired or fermented to different extents (53). Based on our established correlation between glucose uptake and ethanol secretion rate, we asked whether the ability to ferment certain sugars is determined only by the import rate that yeast can achieve for this sugar. Indeed, ethanol production
rates obtained from *S. cerevisiae* grown on galactose (19, 29, 35, 54, 55) compare well with those measured for glucose at the same sugar uptake rate (see Figure 1a). This is also true for other sugars, such as fructose (19), maltose (56-58), arabinose (58), and sucrose (17), when normalized to c-moles (see Figure 1a). In addition, a relationship between import rate and the ability to ferment a particular sugar was also seen for maltotriose (59, 60) and cellobiose (61). Similarly, a strain with an engineered higher galactose uptake rate increased its respiro-fermentative metabolism, with the ethanol production rate increasing linearly with glycolytic flux (54) and *Kluyveromyces lactis* switched to aerobic fermentation of galactose after introduction of the GAL2 gene (62). Along the same lines, a yeast strain lacking invertase activity and with only a limited capacity to transport sucrose into the cell, showed a significantly reduced ethanol production rate (63). The importance of the import rate is further illustrated by the Kluyver effect, which is the inability of a specific yeast strain to ferment a specific disaccharide even though it can be respired and the sugar molecules that make up the disaccharide can be fermented, for example, maltose in *C. utilis* (57). Multiple lines of evidence suggest that the Kluyver effect is strongly dependent on the import rate of the disaccharide (57, 62, 64). For example, Goffrini *et al.* (62) enabled *K. lactis* to grow on raffinose in the absence of respiration by increasing the expression of its transporter. Overall, these observations indicate that the distribution between the respiratory and fermentative pathways is not dependent on the type of substrate but rather on the rate at which that substrate is imported.

The above-mentioned observation that the ethanol excretion rate generally correlates with sugar uptake rate points toward a flux-dependent regulation. It could, however, also be conceivable that it is the growth rate that determines this metabolic behavior, as was suggested, for example by van Hoek *et al.* (23). To test this hypothesis, we plotted the ethanol excretion rates against the growth rates. As we here find absolutely no correlation (cf. Figure 1b), growth rate very likely does not control the rate of ethanol secretion. Further evidence that the growth rate is not the control factor comes from the finding that strains grown in nitrogen-limited chemostats (42) and anaerobic conditions (24, 42), which all result in a different glucose uptake rate at an identical growth rate, maintain a similar relationship between glucose uptake and ethanol production.

Another potential issue could be that the identified correlation could simply also be caused by a physical rate limitation somewhere in metabolism. Indeed it has been argued that the ethanol production is because of an overflow mechanism (23, 65, 66) meaning that there might be a limitation in the TCA cycle or respiratory chain that causes the excretion of ethanol with increasing sugar uptake rates. If this were the case, ethanol excretion would not be the result of an active, flux-dependent regulation. However, there are a number of indications that speak against the overflow hypothesis. (i) If the ethanol excretion were the result of a capacity limitation in the respiratory chain, that is, respiration reaches a maximum level at some point and no further NADH could be respired, then we would expect the oxygen uptake rate to stay at a constant (high) level with further increasing sugar uptake rates. As usually a decrease in the O$_2$ uptake rates is reported with increasing glucose uptake rates (23, 47, 67, 68), it seems
that at least respiration could not be the limiting factor. Further, the highest possible $O_2$ uptake rate is also not reached in aerobic glucose-limited chemostat cultures showing a respiratory metabolism. When yeast is grown in ethanol batch cultures, $O_2$ uptake rates between 11.8 and 13 mmol $O_2$ g$^{-1}$ DW h$^{-1}$ were observed [estimated on the basis of measured ethanol uptake rates (29) and flux variability analysis (69)], while in aerobic glucose chemostat cultures at growth rates between 0.25 h$^{-1}$ and 0.33 h$^{-1}$ (68) $O_2$ uptake rates of only 7.2 mmol $O_2$ g$^{-1}$ DW h$^{-1}$ were measured. (ii) A number of mutant phenotypes indicate that the realized sugar uptake rate and the metabolic mode need to fit together: it was reported that an alcohol dehydrogenase (ADH1) deletion mutant in $S. cerevisiae$ grows poorly in high glucose conditions – a condition requiring fermentation. In contrast, the ADH1 deletion has no phenotype on galactose – a sugar that this strain of $S. cerevisiae$ only respires. Similarly a pyruvate decarboxylase negative (PDC$^{-}$) strain is unable to ferment and grow under high glucose conditions (70, 71). The growth of this strain could be rescued using a directed evolution approach that yielded a strain with a reduced glucose uptake rate (72). In contrast, deletion of the TCA cycle isoenzyme succinate dehydrogenase (SDH1) has no detectable growth phenotype on glucose, but causes an inability to grow on galactose (JC Ewald, T Matt & N Zamboni, unpublished data). Additionally, blocking respiration prevents growth on sugars that are typically respired: a phenotype that can be rescued by increasing the uptake rate of the respective sugar, for example by overexpression of the sugar transporters (62, 64). In our opinion, together these findings point more to flux-dependent regulation than to a simple physical overflow mechanism.

As a result, we propose that there is a regulatory system that (i) measures flux and then (ii) determines whether glucose is utilized by respiration or fermentation (see Figure 2a).

**How could metabolic flux be sensed?**

**Theoretical considerations**

We have seen that the global metabolic phenotype assumed by yeast is dependent on the substrate uptake rate, *i.e.* that there is flux-dependent regulation. This implies that a flux signal needs to be sensed within the cell. Before we discuss how a cell could actually achieve this, we first introduce -from a theoretical viewpoint- two concepts of flux-sensing. A flux through a metabolic reaction is nothing else than a reaction rate. A rate $r$ is a change of a state quantity $x$ between two time points $t$,

$$r \approx \frac{\Delta x}{\Delta t}.$$ 

The first concept for a cell to measure a rate $r$ would require the capability to memorize two states [$x(t = 1)$ and $x(t = 2)$], to measure time and to do mathematical operations in terms of subtractions and a division. It is obvious that cells
cannot do this. However, there is a second possibility of how cells could measure a rate $r$. A rate $r$ can be estimated from a state $x$ (i.e. a metabolite concentration), if the functional dependencies of the system $f$ are known, according to:

$$r = f(x).$$

This concept requires that the cell ‘knows’ the system that generates the state $x$, implying it has a model of the functional dependencies of the system, $f$. For a cell to be able to use this concept, it would be beneficial if there is a simple (ideally linear) dependency between $r$ and $x$ and if no other state variables influence this relationship. Thus, cells that exploit flux-dependent regulation need to have a system that translates a flux ($r$) into a concentration of a biomolecule ($x$). This, for example, flux-signaling metabolite $x$ would then induce flux-dependent regulation (see Figure 2b).

### Clues toward flux-sensing mechanisms in yeast

Where in the metabolic network could the flux be sensed? The fact that the ethanol excretion rate not only correlates with the glucose uptake flux, but with the uptake rates of different sugars (cf. Figure 1a) suggests that the flux sensor needs to be at a point in metabolism that is equally affected by these sugars. The sugars mentioned here converge at either glucose 6-phosphate (G6P) (glucose, maltose, galactose), at fructose 6-phosphate (F6P) (fructose) or at both (sucrose). Thus, the flux-sensing mechanism resides likely below F6P, as also suggested by Moreira dos Santos et al. (73). On the basis of this reasoning, specific transporters or hexokinase 2 (Hxk2) can be excluded, although the latter is frequently suggested to be involved in flux-sensing (9).

![Figure 2](image.png)

**Figure 2.** (a) The collected data in Figure 1a suggest that there is a system (black box) in place that depending on the sugar uptake rate generates different metabolic phenotypes in terms of fermentative and respiratory activity. (b) This system likely comprises of a flux sensor that connects a rate $r$ to a state $x$, which is received by a controller that in turn realizes the necessary regulatory adjustments in a flux-dependent manner.
If we argue here that eventually the glycolytic flux is measured somewhere below F6P, then we will need to check whether there is also a correlation between the sugar uptake rate and the flux between G6P and F6P. A correlation is not necessarily expected, because glucose (or G6P) is also shuffled into the pentose phosphate pathway and into storage metabolism. Nevertheless, data from (partly $^{13}$C based) metabolic flux analyses demonstrate that the glycolytic flux between G6P and F6P linearly correlates with the sugar uptake rate [see Figure 3] for glucose and galactose in different yeast species and independent of the culture conditions employed or the presence of environmental and genetic perturbations (22, 24, 25, 27, 53, 74, 75).

One way to establish a relationship of the kind $r = f(x)$ would be with $x$ being the concentration of a metabolite that would correlate (ideally) linearly with the flux $r$. As metabolite concentrations were found to be highly specific for the limiting nutrient (76), a signaling role for metabolites would not be so far-fetched. Remarkably, the concentration of fructose-1,6 bisphosphate (FBP) seems to correlate with the sugar uptake rate when $S. cerevisiae$ is grown in glucose batch (77) and glucose-limited chemostat cultures with different dilution rates (68) or different cultivation temperatures (39) (see Figure 4a). This correlation even holds for data from other Crabtree positive and negative yeast species (27).

Thus, FBP could be a flux-signaling metabolite, as it was also suggested to be in $E. coli$ (7). Interestingly, when the glucose concentration is suddenly increased in a glucose-limited chemostat culture, or glucose is added to an ethanol-limited chemostat culture, the glucose influx rate increases with a concomitant increase in the concentration of FBP and onset of ethanol excretion (78, 79). This indicates that FBP could not only report the flux in steady state, but also dynamically.

**Figure 3.** Glucose isomerase flux as a function of the sugar uptake rate (normalized to c-mol); data from *Saccharomyces cerevisiae* on glucose batch and chemostat cultures (squares); environmentally and genetically perturbed *S. cerevisiae* on glucose (triangles); *S. cerevisiae* on galactose batch conditions (circles); other yeast species on glucose batch conditions (diamonds).
In contrast, for example, the levels of ATP, ADP, and AMP (27, 68, 80) do not show any clear trend, making it unlikely that the concentration of these metabolites would be the input for the regulatory machinery that controls the activity of the fermentative and respiratory pathways. This conclusion is further supported by the findings from a recent study, in which mitochondrial NAD⁺ carriers were deleted or overexpressed in *S. cerevisiae*. While these perturbations led to altered NAD and ATP levels, all the mutants switched from a fully respiratory metabolism to the respirofermentative one at the same glucose flux as the wild type (10, 81), corroborating the idea that energy cofactors are unlikely to serve as flux signals.

How could information about flux—in the form of a metabolite concentration (for example FBP)—be coupled to the regulatory machinery to finally result in flux-dependent regulation? The first option for such coupling is an interaction of the flux-signaling metabolite directly with enzymes to activate or inactivate their activity (Figure 5), for which a couple of different mechanisms exist (82). FBP, for example, is known to (i) activate pyruvate kinase (83, 84), (ii) have effects on the fructose 2,6-bisphosphate and AMP-mediated activation of phosphofructokinase activity (85), and (iii) inhibit oxidative phosphorylation through strongly inhibiting Complex IV (cytochrome c oxidase) and Complex III (ubiquinol:cytochrome c oxidoreductase) (86).

**Figure 4.** Metabolite levels as a function of sugar uptake rate (normalized to c-mols). (a) Fructose-1,6-bisphosphate concentrations levels, (b) ATP concentration levels, (c) ADP concentration levels, (d) AMP concentration levels. *S. cerevisiae* glucose batch and chemostat cultures (squares), *S. cerevisiae* environmental perturbation (triangles), other yeast species on glucose batch cultures (diamonds).
Alternatively, flux-signaling metabolites could be coupled to the regulatory machinery via interaction with signaling and regulatory proteins, such as kinases or transcription factors. Unlike in *E. coli*, for which about 100 metabolite-transcription factor interactions are known (ECOCYC database (87)), we only know a handful of proteins that link metabolite levels with transcriptional regulation in yeast, for example Gal3p, Put3p, and Bas1p (4, 5, 88). Here, the question is whether we simply do not know more because they are either not as prominent in yeast as they are in bacteria or hard to identify by classical biochemical means and as of today still no high-throughput method exists (89). Although most yeast transcription factors do not have small molecule binding pockets as, for example, the *E. coli* transcription factors (N. Luscombe, pers. commun.), novel metabolite-transcription factor interactions (90) and signaling protein interactions (91) were identified recently. Thus, more might be discovered in the future. Similarly, we are only just realizing that metabolite-binding RNA domains are also present in the genes of eukaryotes (92), which might offer another manner in which flux-signaling metabolites could be coupled to the regulatory machinery of a cell.

In contrast to sensing metabolic flux via flux-signaling metabolites, one could envision that certain enzymes directly sense metabolic flux. It is often speculated that, for example, hexokinase PII (Hxk2p) could do this via conformational changes that accompany the catalysis and that induce localization of Hxk2p to the nucleus or via changes in a signaling complex of which Hxk2p is a component (9). All of this is, however, still elusive and as several sugars are not processed via hexokinase (such as galactose), a flux sensor involving Hxk2 could not represent the full story.

**Toward identifying the regulatory system that controls metabolism in flux-dependent manner**

In response to a sensed glycolytic flux, a cell must be able to direct the flux into either the respiratory or fermentative pathways. Because of the regulatory system’s global and likely complex architecture – overarching multiple cellular levels – we have currently only a few fragmented pieces of evidence about the actual regulatory system:

![Figure 5. Different manners in which flux-information imprinted into concentration levels of flux-signaling metabolites could be coupled to the regulatory machinery.](image-url)
(1) The system needs to be fast, as glucose pulses in glucose- and ethanol-limited chemostat cultures result in an immediate onset of ethanol excretion (78, 79). This indicates that at least part of the system must reside on the fast enzymatic level, for example in the form of metabolite–enzyme interactions. One such action could be the recently identified inhibition of the respiratory chain through FBP (86). Alternatively, fast metabolite–kinase interactions could also be involved in modulation of enzyme activity through phosphorylation. Several new potentially relevant enzyme phosphorylation sites were recently identified (93). With regard to the fact that the system needs to be fast, it is interesting to note that older findings with in vitro systems composed out of a small subset of glycolytic enzymes in a continuous system were found to show different states depending on the glucose feed rate (94).

(2) Redox metabolism has likely a limited role in the system (95). Perturbations in the NAD+ metabolism (which lead to changes in cellular NAD and ATP levels and affect growth rate) did not break the correlation between the sugar uptake and ethanol excretion rate (10). Also increasing respiration or non-respiratory NADH oxidation in S. cerevisiae had only minor effects on the correlation between glycolytic flux and ethanol production rate (81) (the data from these two references are displayed in Figure 1a). Thus, although redox metabolism might be involved to a small extent in establishing overflow metabolism (81), it does not seem to have a major influence on the distribution between respiratory and fermentative metabolism.

(3) Flux-sensing regulation appears to be robust and rigid. As shown above, the relationship between glucose uptake and ethanol secretion rate compares well under many different growth conditions. Various mutations seem to cause at most only relatively minor changes in the sugar uptake rate at which ethanol formation sets in, e.g. (18, 34, 96). However, this amazing robustness causes the regulatory system to be rigid and unable to mediate switching between metabolic modes, when either respiration or fermentation is blocked. This leads to a severe reduction or a complete inability to grow at certain sugar uptake rates (62, 64, 97).

**Conclusion**

Currently, we have only a very limited understanding about the here postulated sensing and regulation system that - in S. cerevisiae and also in many other yeast species- may be responsible for the distribution of flux into the respiratory or fermentative pathways. What we basically know today is the following. (i) The system seems to regulate the metabolic phenotype in a glycolytic flux-dependent manner (cf. Evidence for metabolic flux-dependent regulation). (ii) The concentration of FBP (and likely also other metabolites) is flux-dependent making them ideally suited as potential flux sensors (cf. How could metabolic flux be sensed?). (iii) There are only very fragmented insights into the regulatory system’s characteristics (cf. Toward identifying the regulatory system that controls metabolism in flux-dependent manner).
To eventually obtain a complete systems-level understanding of how ‘metabolic flux controls metabolic flux’, it is clear that next to the classical tools of biological research various systems biology approaches (98) will also be required. For example, high-throughput analytical technologies such as metabolomics and fluxomics will be required for the identification of further potentially existing flux-signaling metabolites, analytical procedures for the detection of novel small molecule–protein interactions and phospho-proteomics to further investigate the relevance of enzyme phosphorylation. Apart from these discovery-driven applications of these modern omics technologies, it will also be required to generate molecule abundance data that after subjecting them to computational top-down analyses will likely be able to extract regulatory interactions. Finally, bottom-up modeling approaches will be required to test hypotheses about whether certain behavior can emerge from the quantitative and dynamic interaction of a select set of molecular players. Here, the grand challenge will be to find the right level of abstraction for the model that will overarch multiple levels of the cell. Only with such a model available will it be possible to ultimately show whether, and if so, how metabolic flux is sensed and used for the regulation.

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