Coordination of microbial metabolism

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Abstract | Beyond fuelling cellular activities with building blocks and energy, metabolism also integrates environmental conditions into intracellular signals. The underlying regulatory network is complex and multifaceted: it ranges from slow interactions, such as changing gene expression, to rapid ones, such as the modulation of protein activity via post-translational modification or the allosteric binding of small molecules. In this Review, we outline the coordination of common metabolic tasks, including nutrient uptake, central metabolism, the generation of energy, the supply of amino acids and protein synthesis. Increasingly, a set of key metabolites is recognized to control individual regulatory circuits, which carry out specific functions of information input and regulatory output. Such a modular view of microbial metabolism facilitates an intuitive understanding of the molecular mechanisms that underlie cellular decision making.

Regulatory circuits
Sets of molecular interactions that have defined information inputs and regulatory outputs.

Metabolic fluxes
The *in vivo* rates of metabolic reactions (or a series of consecutive reactions).

Central carbon metabolism
A core network of about 50 enzymatic reactions that convert carbon nutrients into 'building blocks'.

Regulatory logic
The mapping between the input and output of a regulatory circuit; its characterization can range from signs of interactions to the quantification of governing parameters.

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Metabolism is central to microbial life, and it fuels all cellular activities with building blocks and energy. Despite enormous variation in lifestyles and occupied niches, the fundamental metabolic tasks are highly similar across divergent species (FIG. 1a). For example, all organisms must scavenge nutrients and then coordinate central metabolism, monomer synthesis and macromolecule polymerization for biomass synthesis and proliferation. Furthermore, information about the metabolic state has to be transmitted to other cellular processes to coordinate the availability of nutrients and energy with cellular functions. Given the large and densely connected network of metabolites, enzymatic reactions and regulatory interactions, it is a challenge to understand the intertwined metabolic and regulatory network in its entirety[1–3]. However, it is possible to define individual regulatory circuits[4] that have specific information inputs and regulatory outputs, and then the actual molecular components and mechanisms that control these metabolic modules can be studied using a combination of biological and theoretical approaches (BOX 1). Key to identifying the regulatory circuits that operate across metabolism and its regulatory networks are methods and approaches for the quantification of metabolic fluxes, metabolite concentrations, protein abundances and protein activities (BOX 2).

In this Review, we discuss circuits that have been sufficiently characterized to enable an understanding of two fundamental aspects: the information input, which signals fluctuations in intracellular or extracellular conditions, and the subsequent regulatory output, which results in adaptation or maintenance of metabolic fluxes (FIG. 1b). Specifically, we outline common metabolic tasks and the logic of their regulation, including nutrient uptake, the coordination of central carbon metabolism, the generation of energy, the supply of amino acids and protein synthesis. As these tasks are universal across species, the input and output of specific regulatory modules are often similar for different organisms. Moreover, some metabolites, such as fructose-1,6-bisphosphate (FBP), glutamine and ATP, which occupy key positions in the metabolic network, have emerged as important modulators of metabolism among distantly related species. However, the molecular steps between input and output can vary, as identical regulatory logic can be obtained by different molecular implementations. We focus on the regulation of metabolic tasks in the Gram-negative model bacterium *Escherichia coli* and compare alternative regulation mechanisms in the Gram-positive bacterium *Bacillus subtilis* and the yeast *Saccharomyces cerevisiae*. Thereby, we elucidate common principles of metabolic regulation, highlight recent advances and reveal gaps in our understanding of the role that regulatory circuits have in the cell.

Regulation of carbon uptake and catabolism

Substrate uptake. Heterotrophic microbes, such as *E. coli*, thrive on many carbon and energy sources[5,6]. As permanent expression of all transporters would consume valuable cellular resources and occupy limited membrane space, cells selectively express transport systems on the basis of extracellular and intracellular signals. Extracellular signals are primarily detected by two-component systems, in which a membrane-bound

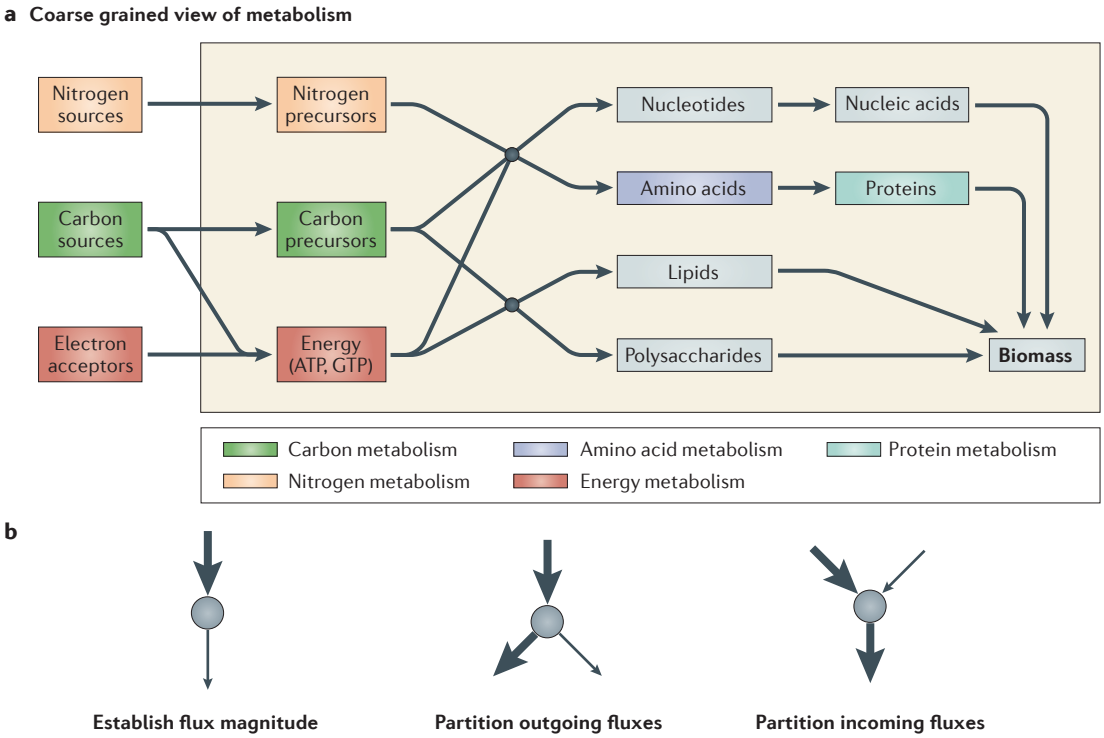


Figure 1 | **Metabolic tasks and the regulation of metabolic fluxes.** **a** | Coarse-grained view of different sectors that compose large parts of metabolism in many bacteria. Microorganisms need to carry out a range of metabolic tasks to ensure a supply of metabolic fluxes through the sectors and thus sustain cell maintenance and growth. All organisms must regulate the uptake of nutrients and coordinate carbon, energy and nitrogen metabolism to balance monomer synthesis and macromolecule polymerization. **b** | Each metabolic task can ultimately be broken down into decisions of establishing the flux magnitude through a linear pathway and the partitioning of incoming or outgoing fluxes at a branch point. Regulatory circuits effect these decisions by modulating enzyme abundance and activity.

Positive-feedback loop
A circuit in which a molecule induces its own production and/or represses its own consumption.

Diauxic growth
The strictly sequential consumption of carbon sources, typically with intermittent adaptation phases.

Catabolite repression
The reduction of alternative nutrient uptake as a result of the presence of a preferred nutrient.

Phosphotransferase system
(PTS). A common bacterial uptake system for sugars, with concomitant phosphorylation in which phosphoenolpyruvate (PEP) is the phosphate donor.

Inducer exclusion
The allosteric inhibition of alternative carbon transporters by the phosphotransferase system in the presence of glucose.

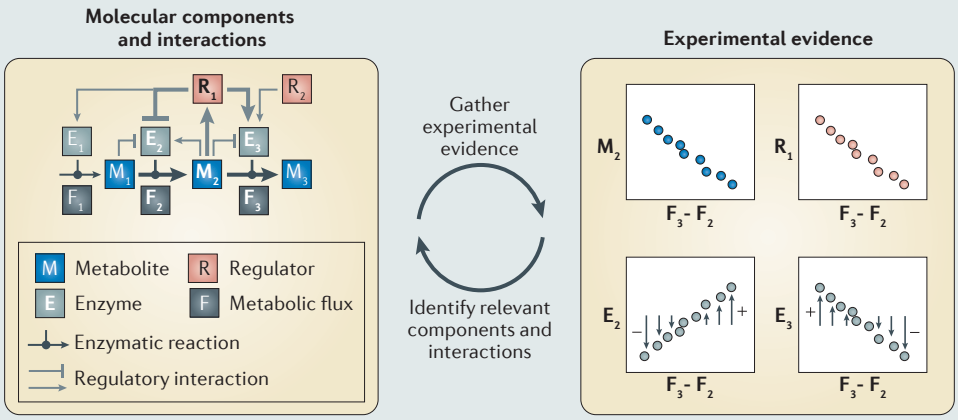
sensor activates a cytoplasmic regulator in response to an external stimulus[7]. About 30 such two-component systems are encoded in the *E. coli* genome, including sensors of phosphate, nitrate[8] and of at least one class of externally sensed carbon sources (phosphorylated sugars[9]).

However, for the vast majority of carbon sources, *E. coli* and many other heterotrophs rely on an intracellular sensor — typically a transcription factor — that both senses the signal and provides a regulatory output[10]. In this one-component internal sensing scheme, transporters and enzymes for the use of various carbon sources are expressed at basal levels, which leads to an increase in pathway intermediates following nutrient availability. This then leads to the upregulation of transporter and enzyme expression via a transcription factor that senses these intermediates. The classic example of this scheme is the repressor LacI of the *lac* operon, which is released from the *lac* promoter following binding of intracellular allolactose[11] (FIG. 2). Other examples include uptake of glucosamine, trehalose, fucose and maltose in *E. coli*[10], and phylogenetic evidence suggests that such one-component sensors are in fact the dominant nutrient-sensing mechanism in prokaryotes[12]. The regulatory logic of the internal sensing scheme results in a positive-feedback loop, which enables small changes in substrate abundance to trigger large transcriptional responses[4]. Thus, the common regulatory principle of these circuits, which enable the demand-based uptake of alternative nutrients, is well understood: the accumulation of a pathway intermediate signals nutrient availability, and this information is then transferred via a transcription factor to increase the magnitude of uptake flux.

Catabolite repression. The positive-feedback circuits that are described above enable carbon source-specific regulation but do not enable prioritization among multiple substrates. The existence of such prioritization is evident from diauxic growth which results from sequential substrate consumption[13]. Several regulatory circuits, which are collectively known as carbon catabolite repression, achieve this prioritization[14] by sensing the presence of preferred carbon sources and reducing the uptake of alternative carbon sources. In *E. coli*, one of the most common preferred carbon sources is glucose, which is transported into the cell via the phosphotransferase system (PTS) (FIG. 2b). When glucose is taken up by the PTS, one of the PTS components, EIIA, is dephosphorylated and directly inhibits transporters for several non-preferred carbon sources. This mechanism is known as inducer exclusion[14,15].

Although inducer exclusion is — at least in some cases — sufficient to achieve carbon source prioritization, *E. coli* encodes an additional system to downregulate the expression of genes that are responsible for the

Box 1 | **Identification of regulatory circuits in metabolism**



The regulatory network that controls cellular metabolism consists of interactions between metabolites, enzymes and regulators. Although the bewildering complexity of the entire network defies comprehensive understanding, more tractable individual circuits can be delineated as modules that carry out concise functions of information input and regulatory effects. A critical step in understanding the function of a given circuit within the larger network is the analysis of its regulatory logic, that is, the relationship between the input and output of the circuit. This process typically involves iterations of hypothesis formulation and the generation of experimental evidence to identify the relevant active interactions that determine the wiring of the circuit. For the example shown in the figure, the regulatory logic that is implemented is the balancing of supply (given by flux F_2) and demand (given by flux F_3) by an integral feedback circuit. An imbalance between supply and demand perturbs the level of the metabolite M_2, which in turn begins to mitigate the imbalance via several regulatory links that affect the enzymes E_2 and E_3.

These regulatory links may be implemented via different mechanisms. Crucial to identifying the relevant interactions is the experimental characterization of the circuit functions, which requires a range of methods to quantify the key measurables (BOX 2).

transport and catabolism of non-preferred substrates. This system centres around the transcription factor Crp, which positively regulates the expression of a number of carbon-uptake systems along with a range of other genes that are involved in carbon catabolism[16,17]. Crp is activated by the intracellular messenger cyclic AMP (cAMP), and cAMP synthesis by the enzyme adenylate cyclase is, in turn, activated by phosphorylated EIIA[15,18]. As described above, phosphorylated EIIA is the dominant form only in the absence of glucose, so by this mechanism, external glucose prevents cAMP synthesis and thus prevents the Crp-mediated activation of alternative carbon-uptake genes.

However, carbon sources that enable high growth rates but that are not transported through the PTS also cause catabolite repression via Crp, which suggests that Crp is sensitive not just to the presence of a set of particular preferred sugars. A partial explanation of this glucose-independent repression was the finding that EIIA phosphorylation depends not only on the availability of glucose but also on the ratio of the central metabolites phosphoenolpyruvate (PEP) and pyruvate[19]. However, several results remain unexplained, which led the authors of a recent review to postulate that an unknown 'factor X' is a regulator of catabolite repression[14]. The demonstration that Crp activity is not only induced by carbon limitation but is also repressed by nitrogen or sulphur limitation suggested that the information that is transferred to Crp is not general carbon availability, but rather the balance between carbon catabolism and the capacity for anabolism[20]. Previous theoretical work had suggested[21] that α-ketoglutarate and other α-ketoacids, such as pyruvate and oxaloacetate, which are the direct carbon precursors for the transamination reaction in amino acid biosynthesis, could be effective regulators of carbon catabolic flux. Indeed, *in vitro* experiments in permeabilized cells showed that α-ketoglutarate and related α-ketoacids inhibited the cAMP-producing enzyme adenylate cyclase, closing the regulatory circuit between carbon availability and Crp activity in an elegant negative-feedback loop (FIG. 2c). In this regulatory circuit, the information about the balance between catabolism and anabolic capacity is transferred to the activity of the transcription factor Crp via the concentration of α-ketoacids, which results in a general shut-down of catabolic gene expression when the ratio of carbon to nitrogen availability is high and induction when this ratio is low[20].

In *B. subtilis*, catabolite repression affects a similar set of genes to those in *E. coli* via the global transcription factor CcpA[22], but this does not depend on cAMP[14]. Although internal sensing of carbon availability seems to activate catabolite repression, the molecular signal is unknown. FBP has been proposed as a signal, but there is only a weak correlation between the concentration of FBP and the degree of catabolite repression of various carbon sources[23]. ATP concentration affects a key step upstream of CcpA activation[24], and recent data show a correlation between GTP and CcpA-dependent transcription[25], which suggests that energy state is a possible input signal. *S. cerevisiae* also exhibits catabolite repression — glucose represses the uptake of alternative carbon

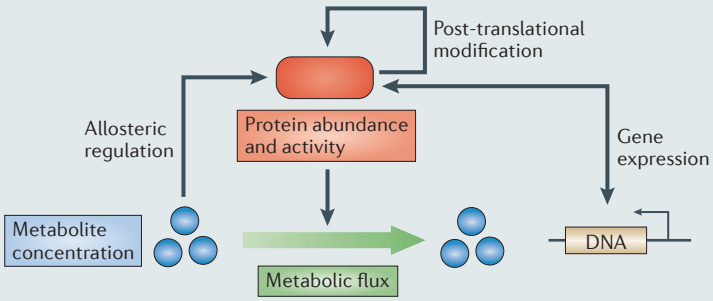
Catabolism
The degradation of complex molecules, such as nutrients, leading to the release of energy.

Anabolism
The energy-dependent formation of building blocks and macromolecules in a cell.

Negative-feedback loop
A circuit in which a molecule represses its own production and/or induces its own consumption.

Box 2 | **Methods and approaches to quantify metabolism and its regulation**

Identification of regulatory circuits in metabolism (BOX 1) requires quantification of underlying molecular components and interactions (see the figure). In this box, we summarize the methods and approaches that are used to estimate the relevant quantities within metabolism and its regulatory networks: metabolic flux, metabolite concentration, protein abundance and protein activity.



Metabolic fluxes

Intracellular fluxes define the metabolic phenotype. Extracellular measurement of substrate uptake and product secretion rates suffices in some cases to constrain possible intracellular flux distributions. Computational models based on predicting fluxes for optimal growth can further guide such efforts[139]. An approach to estimate intracellular fluxes without assuming optimality relies on measuring and model-fitting the propagation of isotopic label (typically ^{13}C) that is provided in the substrate[140,141].

Metabolite concentration

The accuracy and throughput of metabolite concentration measurements (known as metabolomics) have both recently increased owing to improvements in mass spectrometry methods[142,143]. For some metabolites, *in vivo* measurements at the single-cell level are possible using fluorescence resonance energy transfer (FRET) sensors, which enable a fluorescent readout of their conformational change by metabolite binding[144].

Allosteric regulation of protein activity

Essentially all known instances of allosteric regulation have been found by the detailed investigation of purified proteins. Recent efforts to map protein–metabolite interactions on a large scale belong to two major categories. The first category focuses on physical interactions[61,62], whereas the second category attempts to deduce functional activity by correlating metabolite concentration with enzyme activity, ideally during a dynamic transition in which activity changes much faster than gene expression[33,60].

Gene expression

Both RNA-level and protein-level measurements of gene expression have recently continued to progress, as advances have been made in RNA-seq[145], ribosome footprinting[146], fluorescent expression reporters[147] and proteomics[148,149]. In addition, decoding the network of transcription factor–gene interactions has been facilitated by genome-wide quantification of transcription factor–DNA binding by — for example, chromatin immunoprecipitation followed by microarray (ChIP–chip) or chromatin immunoprecipitation followed by sequencing (ChIP–seq) methods[150]. However, a major obstacle remains in decoding transcription factor–metabolite interactions.

Post-translational modifications

Enzyme modifications, such as phosphorylation or acetylation, can drastically affect activity. Identifying the presence of such modifications continues to be a major challenge despite advances in proteomic methods. Considering that about 30% of the yeast proteome is thought to undergo phosphorylation[151] (and similar numbers have been proposed for acetylation in bacteria[152]), an even greater challenge is determining which modifications actually affect protein function[31]. Intermediates of central metabolism were very recently shown to non-enzymatically modify proteins; for example, acetyl-phosphate causes global lysine acetylation[153] following growth arrest, and the glycolytic intermediate 1,3-bisphosphoglycerate causes specific lysine modifications near the active sites of glycolytic enzymes[154] in high glucose conditions. Although these are exciting discoveries, the information that is transferred by these intermediates and the consequences of these modifications remain unclear so far.

sources and causes many other gene expression changes. However, unlike the bacterial systems that are discussed above, yeast relies at least partially, on the external sensing of glucose[26,27]. One consequence of this is a lack of robustness: mutants in which external sensing and transport are decoupled show significant growth defects[28]. However, in predictable environments, in which a small number of external signals are a good indicator of internal changes, external sensing might enable cells to adapt more quickly and could be advantageous[29].

Coordination of central carbon metabolism

Beyond transcriptional regulation. In contrast to the independently operating and mostly well-understood regulatory circuits of uptake pathways, the densely connected network of regulatory interactions within central carbon metabolism has hampered the investigation of its regulatory circuits. Knowing the transcriptional regulatory network is mostly insufficient for understanding central metabolic operations[30], as metabolic control relies heavily on allosteric regulation by metabolite binding and post-translational protein modifications[1,31,32]. The limited relevance of transcription is unsurprising given that metabolism might need to change rapidly — *E. coli* can adapt to environmental changes that reverse central fluxes in a matter of seconds[33,34]. However, methods to deduce metabolite–enzyme interactions or the effect of a covalent modification on protein activity have lagged far behind expression-focused research[1] (BOX 2). Nevertheless, an emerging theme is that cells rely on internal

Allosteric regulation
Regulation of protein activity by remote-site covalent protein modifications (for example, phosphorylation or acetylation) or non-covalent interactions with effector ligands, which change the functional site by the propagation of subtle conformational changes.

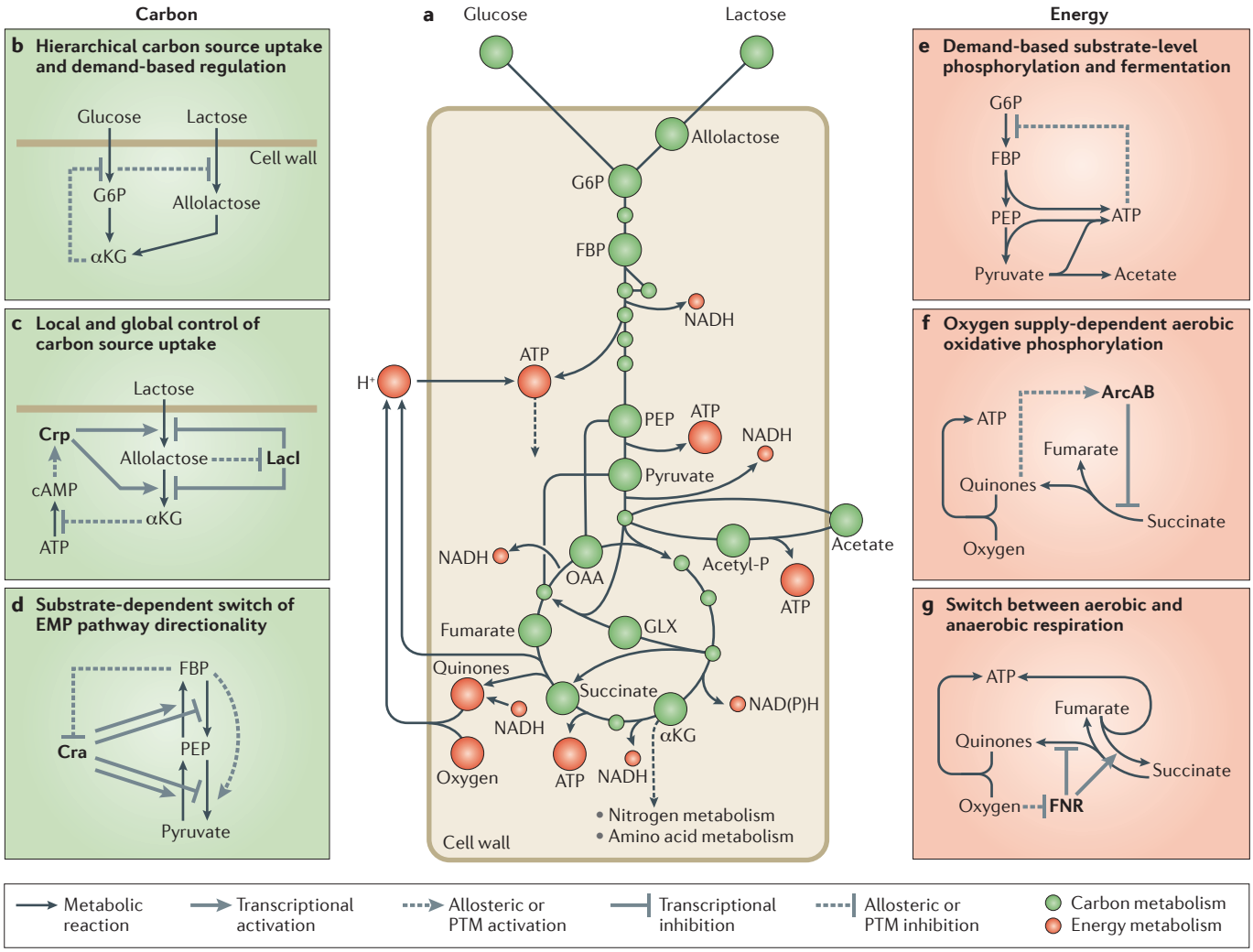


Figure 2 | **Regulatory circuits that control carbon and energy metabolism in *Escherichia coli*.** Schema of central carbon metabolism (**a**). Inset panels display regulatory modules controlling parts of the network: preferential use of glucose as a carbon source (**b**), coordination of local (lactose) and global (carbon supply) signals for carbon uptake by LacI and Crp, respectively (**c**), the fructose-1,6-bisphosphate (FBP)–Cra circuit, which regulates the switch between glycolysis and gluconeogenesis (**d**), control of carbon catabolism via ATP demand (**e**), regulation of respiration by the availability of electron acceptors (**f**) and the oxygen-sensing switch between aerobic and anaerobic respiration (**g**). αKG, α-ketoglutarate; acetyl-P, acetyl-phosphate; cAMP, cyclic AMP; EMP, Embden–Meyerhof pathway; G6P, glucose-6-phosphate; GLX, glyoxylate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PTM, post-translational modification.

signals that are mostly independent of the exact source of carbon, and the crucial cues seem to come from a limited number of central metabolites (TABLE 1).

The FBP switch. A particularly well-characterized example of signalling that uses both allosteric and transcriptional regulation is the substrate-dependent switch that regulates the operation of glycolysis (FIG. 2d). The transcription factor Cra is mostly responsible for repression of glycolytic enzymes, while activating enzymes involved in gluconeogenesis[35,36]. Cra is inactivated during growth on glycolytic carbon sources by binding to the intermediate FBP[37]. It is not immediately obvious why FBP, which is an intermediate of several central pathways, should be more abundant during glycolytic growth. The key insight into the role of FBP as a sensor of glycolytic flux came from the realization that FBP is an allosteric activator of the downstream enzymes pyruvate kinase[38] and PEP carboxylase[39], and thus FBP accumulates until enzyme activity in the lower part of glycolysis matches the upper glycolytic flux[37]. Interestingly, the information transfer from glycolytic flux to the activity of Cra emerges from the topology of the transcriptional and allosteric regulatory interactions of the circuit and seems to be mostly independent of the exact kinetic parameters[40]. Similarly strong dependence on regulatory topology has been shown for many other biochemical networks[41].

The allosteric activation of pyruvate kinase by FBP is conserved even in higher eukaryotes, including humans[42,43], which is suggestive of the potential utility of the flux-sensing circuit that is described above.

Glycolysis
The degradation of sugars to pyruvate, which results in the generation of ATP by substrate phosphorylation reactions.

Gluconeogenesis
The energy-dependent formation of sugars from trioses such as pyruvate.

Table 1 | **Global regulatory metabolites in *Escherichia coli* and the information they transfer**

Metabolite	Information transfer	Key regulatory interaction
FBP	Glycolytic flux[37,39,155]	Enzyme: pyruvate kinase Enzyme: PEP carboxylase Transcription factor: Cra
cAMP	α-ketoacid concentration[20]. Phosphorylation state of PTS system[18]	Transcription factor: Crp
L-glutamine	Nitrogen availability[60,89]	Signalling: GlnBK Transcription factor: NtrC (via GlnBK)
α-ketoglutarate	Ratio of carbon to nitrogen availability for amino acid biosynthesis[20]	Enzyme: EI (PTS component) Enzyme: adenylate cyclase (cAMP forming) Signalling: GlnBK
Other α-ketoacids (for example, pyruvate and oxaloacetate)	Ratio of carbon to nitrogen availability for amino acid biosynthesis[20]	Enzyme: several enzymes in TCA cycle (oxaloacetate) Enzyme: adenylate cyclase (cAMP forming) Transcription factor: PdhR (pyruvate)
L-leucine	Balance of L-leucine production, uptake and protein biosynthesis[110]. General nutrient abundance[110,111]	Enzyme: several steps in branched-chain amino acid biosynthesis Transcription factor: Lrp
ppGpp	Amino acid starvation[118]	Transcription: RNA polymerase Transcription factor: DksA
ATP	Energy starvation[117]	Enzyme: numerous enzymes in metabolism Transcription factor: RpoS via ClpXP Transcription: RNA polymerase (transcription of ribosomal promoters)
Quinones	Balance of respiratory capacity and oxygen supply[77,78]	Signalling: ArcAB

cAMP, cyclic AMP; FBP, fructose-1,6-bisphosphate; PEP, phosphoenolpyruvate; PTS, phosphotransferase system; TCA, tricarboxylic acid.

Curiously, in *B. subtilis*, this feedforward activation seems to be absent[44]. Nevertheless, *B. subtilis* also uses FBP to modulate the activity of several key transcription factors — notably, CcpA and CggR — to control the expression of enzymes in central metabolism[45,46] and, as in *E. coli,* the concentration of FBP in *B. subtilis* correlates with glycolytic flux[25]. The mechanism that is responsible for this correlation in the absence of the pyruvate kinase–FBP interaction is unclear as *B. subtilis* does not encode PEP carboxylase, which is the other FBP-sensitive enzyme in *E. coli*[47]. Apparently, the relationship between FBP and glycolytic flux is also maintained in yeast[48,49], in which FBP activates pyruvate kinase, as in *E. coli*[50]. Although a role in transcriptional regulation has not yet been elucidated for FBP in yeast, other regulatory roles for this metabolite have been proposed, such as the inhibition of respiratory energy generation[51].

The ultrasensitive FBP regulation of pyruvate kinase and PEP carboxylase, both of which act on the common substrate PEP, results in an inverse relationship between FBP and PEP concentrations[39]. PEP accordingly accumulates following glucose depletion, when glycolytic flux reaches zero[52]. This accumulation seems to ensure that there is sufficient PEP to function as a substrate for the phosphorylation of newly internalized glucose through the PTS system when glucose becomes available again[53]. In *S. cerevisiae*, in which glucose phosphorylation does not rely on PEP as a phosphate donor, PEP accumulation might simply be a way to store ATP equivalents[50]. In *S. cerevisiae*, PEP also accumulates following oxidative stress and promotes the production of the redox protectant NADPH in the pentose phosphate pathway by inhibiting the glycolytic enzyme triosephosphate isomerase[54].

Unravelling intertwined circuits. Whereas the regulatory interactions of FBP shed a great deal of light on the regulation of glycolysis, the network of hundreds of other interactions in central metabolism has so far eluded comprehensive understanding. Several molecules, such as PEP, pyruvate, glyoxylate and oxaloacetate, are highly connected; they activate or inhibit many reactions and the activity of several transcription factors[55]. However, it is because of this large number of interactions, many of which might be of little relevance, that little intuitive understanding of their role has emerged. For example, pyruvate, which is an allosteric effector of several glycolytic enzymes[33] and two transcription factors (PdhR and IclR)[56], not only activates its own consumption via the induction of pyruvate dehydrogenase but also regulates genes that are involved in cell division, peptidoglycan synthesis and other distant metabolic reactions[57], and it is unclear which information pyruvate concentration transfers to those processes. Meanwhile, quantitative models of metabolism are hampered by poor characterization of the biochemical parameters. The best predictions of central metabolic fluxes under environmental or genetic perturbations are therefore still based on heuristics or optimality principles rather than biochemical kinetics[58,59] (BOX 2). As complete quantitative characterization is unlikely to be achieved in the immediate future, a useful intermediate step will be to unravel which of the many regulatory interactions are actually active *in vivo* under a given condition and which fluxes they control[1,33,39]. Recent efforts that combine high time-resolution metabolite measurements with computational model selection[33,60] offer some hope and

should be complementary to efforts to map physical interactions[61,62] and analyse *in vitro* enzyme activity[63].

Energy metabolism

The central role of ATP. As the thermodynamic driving force of all cellular processes, biochemical energy — primarily in the form of ATP — is central to life. Two major processes convert the energy in carbon substrates to ATP: these processes are substrate-level phosphorylation (for example, ATP produced in glycolysis) and oxidative phosphorylation (that is, respiration) (FIG. 2e–g). In the latter process, electrons that are obtained from carbon redox reactions are transferred to membrane transport chains, which eventually reduce oxygen or, in more specialized cases, other oxidized compounds, such as nitrate, nitrite or sulphate[64]. This process of respiration yields more ATP molecules per unit carbon source than substrate-level phosphorylation but also requires more proteins. As a result, cells can respond to energy starvation in two ways: they can increase the total amount of carbon catabolism or they can direct more flux to oxidative phosphorylation.

An increase in carbon catabolism is mostly mediated by the aforementioned Crp–cAMP circuit, which senses, among several signals, drops in α-ketoacid concentration, which indicate a lack of carbon relative to nitrogen or other nutrients (a situation that typically leads to energy limitation). However, glycolysis is also sensitive to ATP levels, which are a more direct sensor of energy limitation. Introducing an artificial 'ATP sink' by overexpressing an ATP-dissipating ATPase lowers ATP concentrations and increases glycolytic flux[65,66]. ATP and its congeners ADP and AMP can modulate the activity of many enzymes[65,66,67], but regulation of the reaction between fructose-6-phosphate and FBP is of particular importance. ATP inhibits phosphorylation[68] and AMP inhibits dephosphorylation[69], which leads to increased glycolysis under conditions of low energy charge (FIG. 2e) — a mechanism that is also conserved in humans[70]. However, across a large number of conditions, nucleotide phosphate concentrations and energy charge remain rather constant in *E. coli*, despite wide variation in glycolytic flux[33,71,72], which suggests that other mechanisms also have key roles in the regulation of glycolytic flux.

Transcriptional control of the TCA cycle. Compared with glycolysis, respiration requires many different proteins, from tricarboxylic acid (TCA) cycle enzymes to electron transfer chain components. As such, it is unsurprising that transcriptional regulation has a key role in the control of respiration. Two key transcription factors in *E. coli*, Fnr and ArcA, coordinate the transcription of TCA cycle enzymes with the availability of electron acceptors such as oxygen[73,74] (FIG. 2f,g). Fnr directly senses intracellular oxygen; it represses genes that are involved in aerobic respiration and induces those that are involved in anaerobic metabolism in the absence of oxygen[75,76]. ArcA, as part of the ArcAB two-component system, responds to the redox state of membrane-associated redox carriers in the respiratory chain, namely quinones, which accumulate when respiration becomes limited — for example, as a result of low oxygen availability[77,78]. However, oxygen is not the exclusive regulatory input: transcriptional regulation of TCA enzymes by Crp strongly suggests that carbon source availability has a crucial role[79]. Allosteric regulation, such as α-ketoglutarate inhibition of citrate synthase, which is the first step in the TCA cycle[80], might also transfer carbon-availability information to TCA cycle activity. Thus, *E. coli* seems to use information about carbon, oxygen and energy availability to regulate the TCA cycle and respiration. However, quantitative studies are lacking, and much remains to be learned about how these signals are integrated.

The emerging picture is that the investment of *E. coli* in the energy-efficient pathway of oxidative phosphorylation and the TCA cycle is mostly transcriptionally regulated. Recent evidence from *B. subtilis*[25] and *S. cerevisiae*[81,82] shows a similar picture. A likely explanation for this transcriptional control is the large cost of expressing the many proteins that are required for respiration. As growth rates increase, cells must devote a larger fraction of their proteome to the production of ribosomes[83], hence optimized resource allocation would favour reducing the expression of respiratory enzymes in rapidly growing cells as long as sufficient energy can be provided[84,85].

Nitrogen uptake and metabolism

Uptake of nitrogen sources. Many of the tasks in the regulation of nitrogen uptake and metabolism, such as the detection of various nitrogen sources and their preferential usage, mirror those in carbon metabolism (FIG. 3a). The preferred nitrogen source for *E. coli* in most conditions is ammonium, but a wide range of organic nitrogen-containing molecules can be also used[86]. In parallel to the internal detection of carbon substrates, *E. coli* detects many of these nitrogen sources after they are taken up (via basal expression of their transporters or via non-specific transport) and upregulates enzymes for their transport and catabolism. Such circuits control — for example — the uptake and catabolism of putrescine[87] and sialic acid[88], as well as the catabolism of various amino acids[55].

Signals of nitrogen availability. Whereas *E. coli* uses inducer exclusion to enforce a hierarchy of carbon-source usage, there are only limited examples of such hierarchy in nitrogen-source usage. However, *E. coli* encodes a complex system to transmit information about nitrogen availability[89,90], which is, in some ways, analogous to the previously described Crp–cAMP system that transmits information about carbon availability. At the centre of this system is the signal-transduction protein GlnB (also known as PII), which inhibits the transcription factor NtrC (also known as NRI) , via a phosphorylation cascade (FIG. 3a). NtrC activates the transcription of enzymes for the uptake and catabolism of various nitrogen sources and, intuitively, should be activated only during nitrogen limitation. This information is transmitted by the concentration of glutamine,

Energy charge
A measure of the fraction of nucleotide pools in energetically charged (di- and tri-phosphate) states.

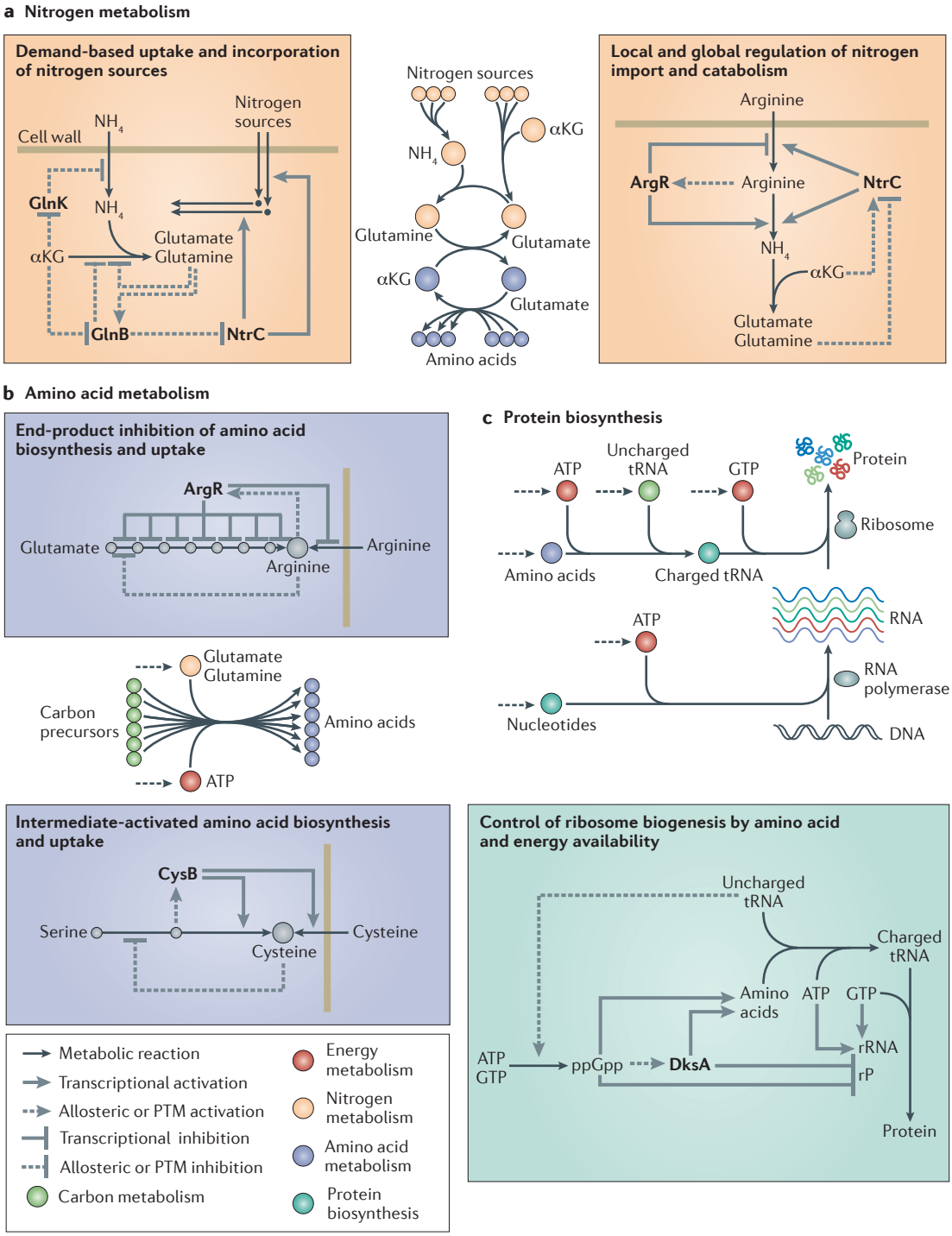


Figure 3 | **Regulatory circuits that control nitrogen metabolism, amino acid metabolism and protein biosynthesis in *Escherichia coli*.** Network components are illustrated schematically and the insets show regulatory modules controlling parts of the network. Regulatory proteins are shown in bold. **a** | α-ketoglutarate (αKG) and glutamine combine to activate the import of nitrogen sources when insufficient nitrogen is available. Although the major functions of GlnB and GlnK (also known as PII proteins) are shown, these two close homologues can fulfil most functions almost interchangeably. The global regulator NtrC functions as a signal of general nitrogen limitation but combines with several local regulators — for example, ArgR as pictured — which report on the availability of particular nitrogen sources. **b** | Amino acids typically control their production by both allosteric regulation of the first committed step and by transcriptional regulation of biosynthetic enzymes — arginine and cysteine are shown here as two examples. Although the regulatory topology differs between the two cases, both networks regulate the transcription of pathway enzymes according to end-product demand. **c** | Uncharged transfer RNA (tRNA) builds up when either amino acids or energy are unavailable and coordinates many processes — in particular, ribosome biogenesis — through the small molecule regulator guanosine tetraphosphate (ppGpp). PTM, post-translational modification; rP, ribosomal proteins.

which is the product of the major ammonium assimilation reaction and is a nitrogen donor for various anabolic processes: high glutamine concentrations convey nitrogen sufficiency and low glutamine concentrations convey nitrogen limitation. Glutamine functions by preventing an inactivating modification (that is, uridylylation) of GlnB, thus inhibiting NtrC[86].

Under high glutamine concentrations, GlnB is also subject to repression by a second signalling molecule, α-ketoglutarate[91,92]. This circuit thus forms an 'OR gate' that activates nitrogen catabolism when glutamine concentrations are low or when α-ketoglutarate concentrations are high[93]. It is unclear which conditions would necessitate such a secondary regulator, but an interesting hypothesis relates to the second major role of GlnB: repression of glutamine synthetase activity[94]. As glutamine synthetase assimilates ammonium and indirectly consumes α-ketoglutarate, repression of GlnB activity by α-ketoglutarate forms a negative-feedback loop by which α-ketoglutarate induces its own consumption (FIG. 3a). However, glutamine synthetase activity is subject to inhibition by many nitrogen metabolism products, such as glutamine itself, several other amino acids and some nucleotides[86], and a physiological role for the described feedback loop has not been observed. Full understanding of this circuit will require quantitative characterization of the role of many inputs.

Nevertheless, these regulatory interactions confirm that α-ketoglutarate is a key molecule that signals the balance between carbon and nitrogen metabolism in *E. coli*, and that it activates nitrogen assimilation and represses carbon assimilation. Repression of carbon assimilation is accomplished via the already discussed Crp–cAMP loop, but is also achieved via the direct inhibition of glucose uptake by the PTS system[95]. Aside from the GlnB regulation that is discussed above, α-ketoglutarate has a further role in nitrogen assimilation, as it activates ammonium uptake via binding to the protein GlnK, which is a homologue of GlnB[96], and relieves its repression of the high-affinity ammonium transporter AmtB. A recent model suggests that this activation of AmtB, which is coupled to a high energy cost of transport, occurs at the precise level that is necessary to maintain the internal ammonium concentration[97].

Like *E. coli, B. subtilis* and *S. cerevisiae* both rely heavily on glutamine concentrations to regulate the activity of the global transcription factors that are responsible for the uptake and catabolism of various nitrogen sources[98,99]. The respective molecular implementations essentially bear no relation to each other, but the regulatory logic of using glutamine as a signal of nitrogen sufficiency or limitation is conserved. *S. cerevisiae* even features clear catabolite repression of non-preferred nitrogen sources via the preferred nitrogen sources — glutamine, asparagine and ammonia[100]. Although less attention has been focused on α-ketoglutarate, GlnB homologues (that is, PII proteins) are found throughout bacteria as well as in archaea and plants[93], and the mechanism of their regulation by α-ketoglutarate seems to be widely conserved[101].

Transcriptional attenuators
RNA structures that cause transcriptional termination only in the presence of a metabolic end-product, such as an amino acid.

Amino acid uptake and metabolism

In the previous sections we discussed how cells regulate the conversion of nutrients into metabolic intermediates, which then have to be converted into monomers, such as amino acids, nucleotides and lipids for macromolecule synthesis. As an example, in this section we discuss the coordination of individual amino acid biosynthesis and degradation pathways (FIG. 3b). This coordination is mostly achieved by end-product inhibition, which is a ubiquitous regulatory mechanism that balances the production of a specific amino acid with its demand but minimally affects the rest of metabolism[102,103]. All 20 amino acids in *E. coli* have either been shown to inhibit the first committed step in their synthesis via allosteric regulation[55] or, in the case of single-reaction pathways, can be assumed to do this via product inhibition. This mechanism ensures a rapid increase in synthesis in response to a higher demand, or repression of synthesis in response to an excess supply. As the inhibition typically affects only the branch of the pathway that is specific to the particular amino acid, parallel branches can mostly be tuned independently. To a large extent, similar principles also govern the transcriptional regulation of amino acid biosynthesis: at least ten amino acids negatively regulate the transcription of their own biosynthesis pathways, either via transcription factors[104] or transcriptional attenuators[105]. However, in several cases, transcriptional regulation is mediated by a factor that binds to a pathway intermediate that builds up when allosteric regulation of the first step is relieved[106]. Nevertheless, in each case the regulation follows the straightforward logic of increasing flux when amino acid usage exceeds supply, and hence the transferred information is the balance between supply and demand.

Such pathway-specific transcriptional regulation can be co-opted to regulate degradation as well as synthesis. For example, the transcription factor ArgR, which binds to arginine to represses arginine biosynthesis enzymes when arginine is abundant[107], can also activate arginine degradation enzymes[108] (FIG. 3a,b). Such regulation is also subject to the NtrC regulation that is described above, thus integrating a global sensor of nitrogen demand with a local sensor of arginine availability.

Nevertheless, not all parts of the amino acid metabolism regulatory network are clearly separated into specific branches. There is crosstalk at both the allosteric[109] and transcriptional[104] levels, whereby amino acids affect not only their own synthesis but also the synthesis of other amino acids. Moreover, some amino acids function as global regulators, affecting the activity of transcription factors that target hundreds of genes. One such transcription factor in *E. coli* is Lrp, which binds to leucine but regulates hundreds of genes that are involved not only in amino acid biosynthesis but also in preparation for stationary phase[110,111]. Curiously, in *B. subtilis*, the global regulator CodY regulates many genes that are involved in the transition between growth and starvation and is also sensitive to levels of branched-chain amino acids, such as leucine[112]. The yeast targets of rapamycin (TOR) complex, which similarly regulates many genes

related to growth and starvation, also seems to be particularly sensitive to leucine levels[113]. No concrete theory has been proposed for why leucine should be such a commonly used signal of general starvation.

Protein synthesis and growth

One of the endpoints of metabolism is the assembly of amino acids into proteins (FIG. 3c). In a fast-growing cell, protein synthesis and ribosome production account for most nutrient and energy consumption. However, as fast growth can require the devotion of up to 75% of cellular transcription to the production of ribosomes[114], such a programme would be highly deleterious when nutrients are limited[83,115], and thus *E. coli* devotes resources to ribosome biogenesis only when resources for protein synthesis are abundant. Making this decision requires the integration of several metabolic signals, and *E. coli* uses the availability of energy sources (such as ATP and GTP) and amino acids, which are the major substrates of protein synthesis, to determine the rate of ribosome biogenesis. The concentrations of ATP and GTP directly influence transcription at ribosomal RNA (rRNA) promoters via the availability of initiating nucleotides[116]. Moreover, in starvation conditions, a decrease in ATP levels also indirectly affects ribosome biogenesis by inhibiting the degradation of the stress sigma factor RpoS, thereby redirecting the transcriptional machinery from ribosomal genes to stress-response genes[117]. The signal of amino acid availability is channelled by guanosine tetraphosphate ((p)ppGpp), the synthesis of which is activated by the presence of uncharged (lacking amino acid) transfer RNA (tRNA) molecules via the allosteric regulation of the enzyme RelA[118]. The small molecule (p)ppGpp can then repress rRNA transcription both directly[119] and via the binding of the transcription factor DksA[120]. Although there have been few quantitative studies of the precise input function, it is probable that the combination of these two inputs enables the production of rRNA only in conditions in which both ATP and amino acids are available. Similar control is exerted over ribosomal protein expression[121]. The synthesis of some amino acid biosynthesis enzymes is also induced by (p)ppGpp, thus it functions as a global regulator in addition to regulating pathway-specific signals.

B. subtilis also relies on (p)ppGpp to sense amino acid availability via the concentrations of uncharged tRNA. However, unlike in *E. coli*, (p)ppGpp does not directly affect the transcription of rRNA, but rather lowers the pool of GTP, both by draining GTP for ppGpp synthesis and by inhibiting a key enzyme in the GTP biosynthesis pathway[122,123]. The decreased GTP concentration then lowers transcription initiation rates at rRNA promoters[124]. Unlike *E. coli*, which uses both ATP and GTP as initiating nucleotides for rRNAs, *B. subtilis* exclusively uses GTP, which makes its rRNA synthesis mostly independent of ATP[124]. The physiological importance of this is unclear — ATP could function using alternative mechanisms, such as induction of the σ^B stress response[125], or ATP and GTP could sense different aspects of the energy state.

In *S. cerevisiae*, accumulation of uncharged tRNA leads to the activation of the kinase Gcn2, which phosphorylates the translation initiation factor eIF2α[126]. This immediately slows translation in general, but specifically increases translation of the transcription factor Gcn4, which activates the expression of many amino acid biosynthesis enzymes[126]. There is no known link between Gcn2 and ribosome biogenesis. Instead, ribosome biogenesis is mostly controlled by two pathways in parallel: the protein kinase A (PKA) pathway and the TOR complex[127]. There is still some mystery to the molecular signals that are responsible for the activation of both pathways, but there is strong evidence that the TOR complex is directly repressed by the absence of amino acids, which inhibits ribosome biogenesis via a phosphorylation cascade[128]. The PKA pathway seems to directly respond to the presence of glucose[129] and thus integrates information from external sensors of nutrient availability.

The reason that superfluous ribosome biosynthesis would be deleterious is that cells can only modulate their total protein allocation, taking resources from the expression of one protein and devoting them to another[20,83]. Given that ribosomal proteins and metabolic enzymes are the major protein fractions at high growth rates[130], decreased ribosome synthesis would enable increased enzyme synthesis. Conversely, this constraint of total protein allocation is a plausible explanation for why rapidly growing cells primarily rely on glycolytic energy generation and not on the more protein-intensive respiration[20,84]. Complementary to the common perception of protein synthesis regulating metabolism, *E. coli* thus coordinates proteome partitioning between ribosome and metabolic enzyme synthesis via metabolic signal-dependent transcription factors — for example, by Crp and the global carbon/nitrogen availability reporter α-ketoglutarate[20].

Discussion

The regulatory network that controls metabolism is daunting, even for supposedly simple microorganisms. Hundreds of regulators can influence metabolic functions via the transcriptional regulation of enzyme expression, post-translational enzyme modification or allosteric binding. To understand the operation of this network, it is vital to decode the information that is transmitted by the concentrations of metabolites that interact with enzymes and regulators. About one-third of the 200 transcription factors in *E. coli* are known to[55], and many more are expected to[131], bind to metabolites. Similar numbers of metabolite effector–regulator interactions are known for *B. subtilis*[132]. In yeast, many fewer interactions of metabolites with transcription factors[133] and kinases[134] are known, but physical-interaction mapping is starting to reveal an extensive network[61,62].

In this Review, we have only touched the 'tip of the iceberg' of these regulatory interactions, which control the metabolic response to the entire range of environmental fluctuations. Nevertheless, the systems that we have described are responsible for a large part of the robust operation of metabolism, by controlling the global response to nutrient availability and environmental cues that affect growth. Throughout this Review, we

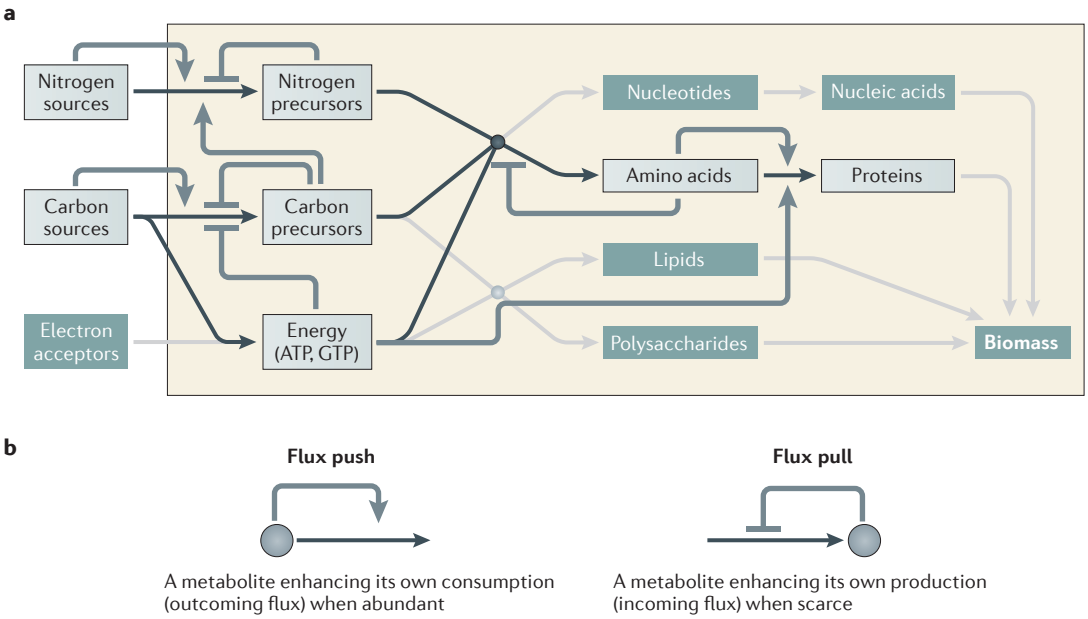


Figure 4 | **The high-level logic of *Escherichia coli* metabolic regulation.** The specific regulatory interactions shown in FIGS 2,3 are summarized and overlaid on the coarse-grained metabolic network shown in FIG. 1. **a** | Coordination within sectors is obtained by a high-level combination of activation and inhibition by means of different molecular implementations. Individual modules maintain homeostasis and inter-pathway balance by activating their consumption and repressing production, either directly or indirectly. **b** | The logic that is implemented by regulatory circuits across sectors can be summarized by two major motifs implementing negative feedback loops: the 'flux push' motif, by which a high abundance of a metabolite activates its own consumption, and the 'flux pull' motif, by which a low abundance of a metabolite activates its production.

have observed that *E. coli* (and presumably other heterotrophic bacteria) relies on relatively few intracellular signals that report on key aspects of the metabolic state (TABLE 1). These global, but also many of the locally acting, regulatory metabolites seem to function both in rapid allosteric enzyme regulation and in comparatively slow transcriptional regulation. An attractive theory is that cells use allosteric enzyme regulation to control flux magnitudes and to correct imbalances and transcriptional regulation to manage resource allocation in response to, or in preparation for, environmental changes.

An emerging theme is the coordination of local and global signals. For example, the uptake of specific carbon sources is subject not only to regulation that senses the presence of that carbon source but also to regulation that senses the overall carbon demand, which then integrates information about the availability of other nutrients and overall growth suitability. One can think of this coordination as a series of nested loops in which general signals of the cell state control large modules of metabolism and local signals regulate the flux within the module. The crucial and final outer loop of such nested feedback loops is the total capacity of the cell — for example, to synthesize its proteome, which must be optimally distributed between ribosomes, metabolic enzymes and other proteins. In many cases, no special mechanisms are required for coordination. Under some simple assumptions, substrate activation and product inhibition of pathway modules, such as the inhibition that is exemplified in amino acid biosynthesis pathways, is sufficient for efficient metabolism[21]. The ubiquity of this architecture, even for large metabolic modules, is evident in FIG. 4. Imbalances between pathway supply and demand are reflected in substrate and end-product concentrations, and the negative-feedback loop can quickly and stably rebalance fluxes.

Although the nested-feedback architecture aids intuitive understanding of metabolic regulation, it also causes considerable difficulties in experimental studies, as perturbations that affect the global cellular state, such as the growth rate, will feed back to virtually all metabolic operations, obscuring the interpretation of readouts such as gene expression. As such, one of the key methodologies, which has been made explicit in several studies[20,135], is to monitor the relationship between expression and growth rate using several different types of metabolic perturbations. Alternatively, in situations in which the effect of growth rate on expression is well understood, one can also use mathematical models to separate this effect from more specific gene regulation[107,136–138].

Throughout this Review, we have focused on clearly delineated regulatory circuits, attempting to illustrate how quantitative measurements of cellular components, in combination with growth physiology, revealed the logic of information transfer and functional execution of flux adaptation. Cells have evolved to be reliant on relatively few components to report on their global metabolic status and mount appropriate responses. Hence, understanding the relationship between the environment

and these intracellular signals can be a fruitful source not only of intuitive understanding but also of hypothesis generation about the underlying molecular mechanism. This simplicity of the reliance of cells on few components within the complexity of the many co-occurring regulation events can also be exploited to infer the metabolic status in a given environment from measurements of those key control variables. As the described regulatory circuits quantitatively capture many of the dynamic properties of metabolism, we envisage them in the long run as modules in larger, coarse-grained and multi-scale models, eventually capturing the whole cell[3], to support a new generation of models that will generate fundamentally new, experimentally testable predictions.

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