Understanding carbon catabolite repression in *Escherichia coli* using quantitative models

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Carbon catabolite repression (CCR) controls the order in which different carbon sources are metabolized. Although this system is one of the paradigms of the regulation of gene expression in bacteria, the underlying mechanisms remain controversial. CCR involves the co-ordination of different subsystems of the cell that are responsible for the uptake of carbon sources, their break-down for the production of energy and precursors, and the conversion of the latter to biomass. The complexity of this integrated system, with regulatory mechanisms cutting across metabolism, gene expression, and signaling, and that are subject to global physical and physiological constraints, has motivated important modeling efforts over the past four decades, especially in the enterobacterium *Escherichia coli*. Different hypotheses concerning the dynamic functioning of the system have been explored by a variety of modeling approaches. We review these studies and summarize their contributions to the quantitative understanding of CCR, focusing on diauxic growth in *E. coli*. Moreover, we propose a highly simplified representation of diauxic growth that makes it possible to bring out the salient features of the models proposed in the literature and confront and compare the explanations they provide.

CCR and mathematical modeling

All free-living bacteria have to adapt to a changing environment. Specific regulatory systems respond to particular stresses, but the most common decision bacteria have to make is the choice between alternative carbon sources, each sustaining a specific, maximal growth rate. Many bacteria have evolved a strategy that consists in utilizing carbon sources sequentially, in general favoring carbon sources that sustain a higher growth rate. As long as a preferred carbon source is present in sufficient amounts,

the synthesis of enzymes necessary for the uptake and metabolism of less favorable carbon sources is repressed. This phenomenon is termed CCR and the most salient manifestation of this regulatory choice is diauxic growth (Figure 1) [1–6].

CCR, occupying such a central position in the regulation of bacterial metabolism, has been intensely studied for more than 50 years. The underlying regulatory system involves a complex interplay between metabolism, signaling by metabolites and proteins, and the regulation of gene expression, all in the context of global constraints on cell physiology. However, the precise role of the different mechanisms that have been identified remains controversial [2]. In this review we expose the fundamental regulatory logic of CCR and we summarize different mechanisms that produce diauxic growth. Even though the regulatory logic is common to all bacteria, we focus on *Escherichia coli* for specific examples.

Owing to the complexity of the regulatory networks in the cell, an intuitive understanding of CCR is virtually impossible. To explain how the observed behavior of a bacterial cell emerges from networks of biochemical reactions and regulatory interactions, and predict the response of this system to specific experimental perturbations, mathematical models have been found useful [7,8].

A variety of models has been proposed for CCR, focusing on different aspects of the phenomenon. Flux balance models predict the distribution of metabolic fluxes that maximize the growth rate in the presence of a mixture of carbon sources [9,10]. An extension of the flux balance models also takes into account constraints imposed by the limited amount of resources available to the cell (density of transporters in the cell membrane, translational capacity, macromolecular crowding, etc.) [11–13]. The resource allocation view is also present in models that weigh the growth benefit of gene regulation against the costs of producing the necessary regulatory proteins [14–16]. Mechanistic models focus on enzyme induction, in other words, the regulation of the expression of enzymes needed to metabolize a particular carbon source. Moreover, they include signaling events that inhibit the activity of transporters of less-preferred sugars in the presence of the

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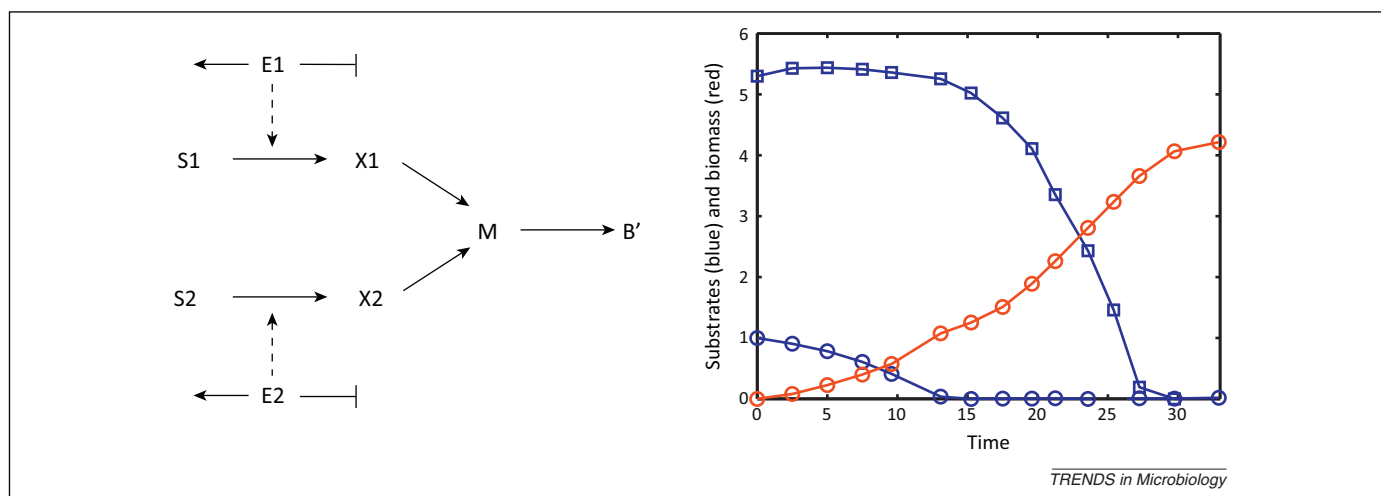http://dx.doi.org/10.1016/j.tim.2014.11.002

Figure 1. Carbon catabolite repression (CCR) and diauxic growth. When in the presence of two different growth substrates, the bacterium first metabolizes via enzyme E_1 the substrate sustaining the highest growth rate (S_1). After exhaustion of the preferred substrate, the enzyme E_2 necessary for the utilization of the second substrate (S_2) is synthesized, leading to a temporary growth lag, after which slower growth resumes on S_2. Both substrates are converted by catabolic reactions into common precursor metabolites, M, via different intermediates, X_1, and X_2. These precursors are used in anabolic reactions for the generation of an internal biomass compartment, B'. Experimental data for glucose (S_1, blue circles), lactose (S_2, blue squares), and biomass (B', red circles) in the right panel are taken from [17]. CCR refers to the different mechanisms that bring about the above-mentioned changes in enzyme and metabolite levels and metabolic fluxes. A variety of regulatory mechanisms completing the simple reaction scheme are considered in this review.

preferred carbon source, a mechanism termed inducer exclusion (Figure 2). Integrated models attempt to combine these mechanisms as well as other metabolic regulation mechanisms into a large model, and are usually formulated as systems of ordinary differential equations (ODEs) [17–19]

We review here these different modeling approaches and assess their capacity to predict the hallmark feature of CCR, diauxic growth. While CCR is ubiquitous in microorganisms [1,2], almost all modeling studies have focused on *Escherichia coli*, the first organism for which molecular mechanisms involved in CCR have been identified in great detail. We propose a highly simplified representation of diauxic growth (Figure 1) to explain and compare the salient features of the models that have been proposed in the literature. We show that the overall logic of diauxic growth can be explained by all modeling approaches in specific situations or for particular combinations of carbon sources. We argue that the approaches are complementary in the sense that CCR involves regulation at both the metabolic and gene expression levels, and both specific regulatory mechanisms and global physical and physiological constraints. To answer some of the unresolved questions about CCR, we therefore need to combine elements from several of the existing models.

Flux balance view

Bacteria are commonly assumed to have optimized the functioning of their metabolism under selective pressure from the environment. While the objective that may be optimized is the subject of debate [12,20–22], it is usually proposed that bacteria maximize their growth rate, sometimes at the expense of biomass yield or ATP. For example, fast growth on glucose leads to acetate secretion, a phenomenon known as overflow metabolism [23–25]. From this perspective, the distribution of fluxes in the metabolic network, and notably the rate of uptake of carbon sources

in a given environment, would be selected so as to favor maximal growth. Can this optimality argument, which abstracts from the actual molecular mechanisms regulating the fluxes, be exploited to explain the sequential uptake of carbon sources by a bacterial cell?

The methodological framework in which this question has been developed is flux balance analysis (FBA) [9,10]. Based on a stoichiometry model of the metabolic network and constraints on metabolic fluxes, FBA looks for flux distributions in the network that optimize the growth rate or another objective function. Box 1 summarizes the mathematical and computational background of FBA, which has been used in a wide range of applications in microorganisms [26]. In particular, using genome-scale reconstructions of metabolism, FBA has been shown capable of accounting for a range of growth-related phenomena in *Escherichia coli* [27,28].

FBA provides a steady-state picture of metabolism. However, the predicted optimal flux distributions can be combined with a dynamic model of the concentrations of external metabolites and biomass, describing how the latter evolve due to the uptake rate of substrates, the secretion rate of byproducts, and the growth rate of the cells. This extension, known as dynamic flux balance analysis, makes it possible to simulate processes such as batch growth of a bacterial population in the presence of multiple substrates [29,30].

Dynamic FBA can reproduce diauxic growth on glucose and acetate in *E. coli*. Given a genome-scale stoichiometry model and (measured) capacity constraints on the glucose and oxygen uptake rates [31], cells are predicted to start growing on glucose. Above a specific biomass density, when the oxygen uptake rate no longer allows all glucose entering the cell to be completely oxidized, acetate overflow occurs. When all glucose has been consumed, acetate is taken up and converted into biomass, at a lower rate (Figure S1 in the supplementary material online). Notice that, in this case,
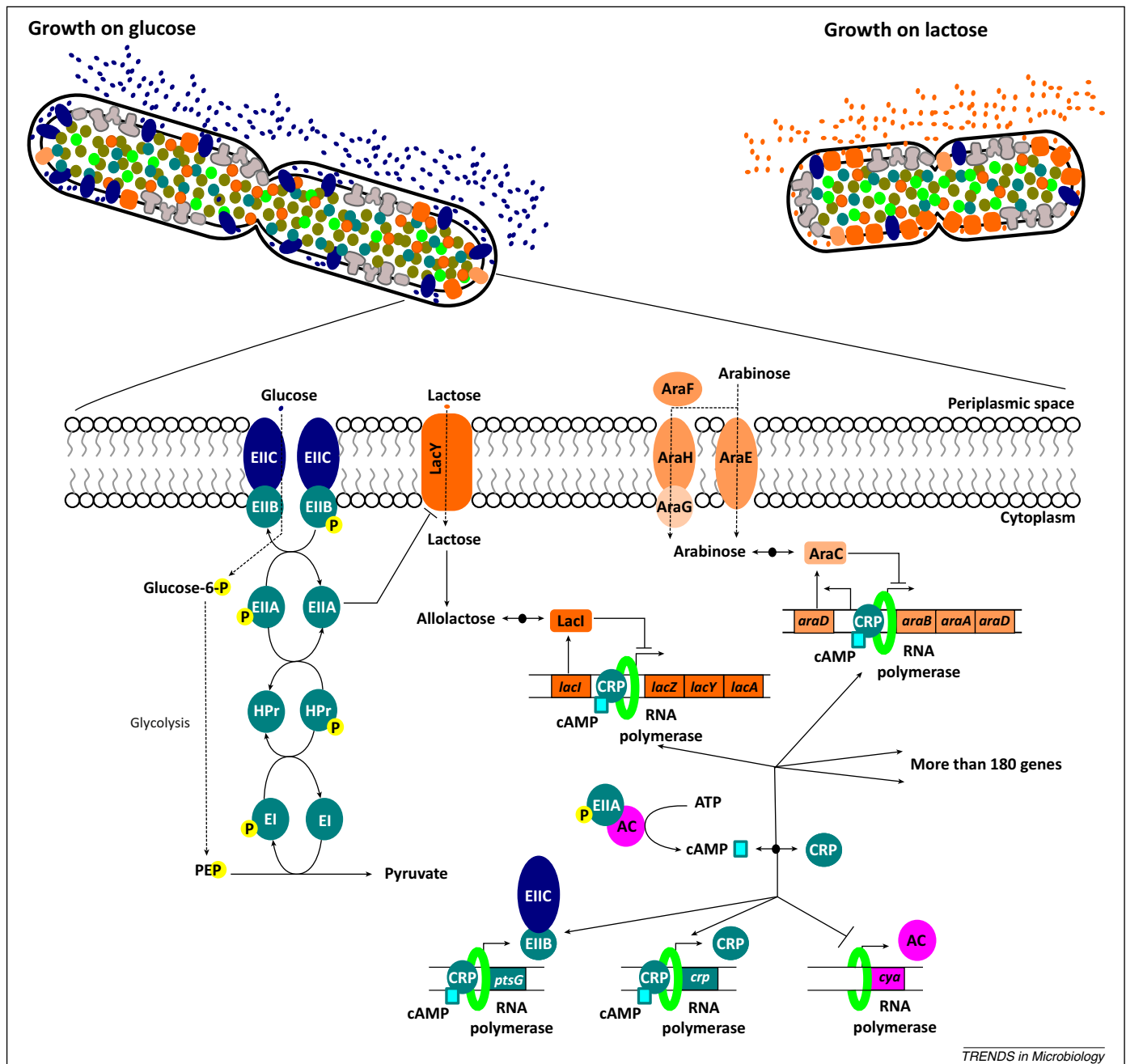
Figure 2. Global physiological effects and regulatory mechanisms involved in carbon catabolite repression (CCR) in *Escherichia coli*. **(Above)** The cytoplasm of the cell contains macromolecules at a high concentration, constraining the total intracellular volume available for metabolic enzymes. The membrane is occupied with transporters and respiratory chain proteins. **(Below)** Zoom view of the cytoplasmic membrane and the cytoplasm. Glucose is transported and phosphorylated (P) by the phosphotransferase system (PTS) composed of proteins EI, Hpr, EIIA^{Glc}, and EIIBC^{Glc}. The phosphate is donated by phosphhoenolpyruvate (PEP) while the latter is converted into pyruvate. Dephosphorylation of EIIA^{Glc} during glucose uptake leads to inducer exclusion, the inhibition of the uptake of non-PTS carbon sources such as lactose. The phosphorylated EIIA^{Glc} enzyme activates the adenylate cyclase (AC), which converts ATP into cAMP. This metabolite binds to the transcription factor Crp, a regulator of more than 260 genes, among which are the genes included in the *lacZYA* and *araBAD* operons, the gene *cya* coding for the adenylate cyclase, the gene crp encoding the cAMP receptor protein *Crp*, as well as the gene *ptsG* coding for the PTS subunit EIIBC^{Glc}. The Crp·cAMP complex stimulates transcription by stabilizing the binding of RNA polymerase to the promoter region. Transcription inhibition occurs by competition for promoter binding between Crp·cAMP and RNA polymerase. In the absence of inducer (allolactose and arabinose), enzyme induction does not take place owing to repression of the *lacZYA* and *araBAD* operons by LacI and AraC, respectively. The example illustrates growth of *E. coli* cells on glucose (upper left) and lactose (upper right). The growth rate is slower and the cell size smaller during growth on lactose than on glucose (cells are not drawn to scale, see [91] for measured cell volumes). In addition, lactose permeases rather than PTS proteins EIIBC^{Glc} are more prevalent in the inner membrane when growing on lactose.

diauxic growth does not require any regulatory constraints (enzyme activity or enzyme expression).

Interestingly, the same model fails to predict glucose–lactose diauxie (Supplementary Information I in the supplementary material online). When growing on a mixture

of glucose and lactose, the cells are predicted to take up these carbon sources simultaneously, contrary to what is observed experimentally [3,4,17]. This suggests that, whereas the glucose–acetate diauxie could be reproduced from metabolic constraints alone, regulation is necessary

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Box 1. Modeling CCR

Bacterial metabolism is conventionally viewed as a system of biochemical reactions that convert external substrates into biomass and byproducts. This system can be modeled by coupled ordinary differential equations (ODEs) describing how the reactions, occurring at a specific rate r_j, change the metabolite concentrations c_i over time. The stoichiometry matrix N couples the intracellular metabolites to the reactions, by indicating which metabolites are produced and consumed in the reaction, and at which relative ratios. The models also include dilution by growth, proportional to the growth rate μ:

$$\underline{\dot{c}} = N\underline{r} - \mu\underline{c}. \tag{I}$$

The simple metabolic network fueling growth from two different substrates, shown in Figure 1 in main text, can be written in the above form by defining $\underline{c} = [X_1, X_2, M]'$, $\underline{r} = [r_1, r_2, r_3, r_4, r_5]'$, and

$$N = \begin{bmatrix} 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 1 & 1 & -1 \end{bmatrix}.$$

Notice that at this level of description the dependency of the reaction rates on metabolite and enzyme concentrations is not explicitly taken into account. Enzymes E_1 and E_2 are assigned to reactions r_1 and r_2.

The model for internal cellular processes is coupled to differential equations describing substrate (S_i) uptake and biomass (B) growth over time:

$$\dot{S}_i = -r_{si} B, \quad S_i(0) = S_{i0}; \quad \dot{B} = \mu \beta, \quad B(0) = B_0 \tag{II}$$

The steady state of the stoichiometry model or flux balance equation given by Equation I (while usually neglecting the growth dilution term) is underdetermined because there are generally more reactions than metabolites. Additional constraints on the fluxes can be defined, based on measurements of uptake or secretion fluxes, limits on enzyme capacity, or thermodynamic constraints.

Flux balance analysis (FBA) aims at selecting solution(s) of the steady-state equation that optimize a particular criterion such as biomass production or ATP production. While classical FBA considers the network at one specific (quasi-)steady-state, dynamic FBA allows the (quasi-)steady state to vary over time as a function of changing substrate concentrations and other growth conditions. At each time-point, the metabolic fluxes are defined as the solution(s) of a flux balance optimization problem and the concentrations of external substrates, products, and biomass evolve in accordance with the optimized exchange fluxes.

Taking into account kinetic expressions for reaction rates r_j as a function of the intra- and extracellular concentrations leads to a fully dynamic model. Usually, as in dynamic flux balance analysis, central metabolism is assumed to adapt quickly to changes in external substrate and enzyme concentrations. As a consequence, intracellular metabolites X_i, M are at quasi-steady-state, which leads to a model in the form of a differential algebraic (DA) system. Scaling of equations (lower-case characters for the state variables) is an appropriate method to reduce the number of parameters and to bring the system onto a defined time-scale (here based on the maximal uptake rate of the first enzyme). Using Michaelis–Menten and first-order kinetics, the scaled model for the simple example of Figure 1 in main text reads as follows:

ODE :
$$\dot{s}_1 = -\frac{e_1 s_1}{1+s_1} b; \quad \dot{s}_2 = -k_s K_s \frac{e_2 s_2 g_2}{1+s_2} b; \quad \dot{b} = \mu b$$

$$\dot{e}_1 = k_{e1} f_1 - (\mu + k_{d1}) e_1; \quad \dot{e}_2 = k_{e2} f_2 - (\mu + k_{d2}) e_2; \quad \dot{b}' = m - (\mu + k_{di}) b';$$

algebraic :
$$x_1 = \frac{e_1 s_1}{1+s_1}; \quad x_2 = \frac{e_2 s_2}{1+s_2}; \quad m = x_1 + k x_2; \quad \mu = \frac{e_1 s_1}{1+s_1} + k_s Y_s \frac{e_2 s_2}{1+s_2}$$

Terms f_1, f_2 and g_2, which may be functions of other model variables, allow the regulatory properties of the network to be taken into account.

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in other cases. The FBA approach has been extended to account for regulatory mechanisms, notably by integrating rules for determining the expression of enzymatic genes. For example, in the presence of glucose in the growth medium, the *lacZYA* operon is repressed, thus constraining the flux through the reactions catalyzed by the transporter and enzymes encoded by the operon. The additional constraints change the geometry of the flux cone and thus the predicted optimal solutions by FBA [32]. This so-called regulatory FBA is capable of predicting glucose–lactose diauxie and a large number of other growth phenotypes of *E. coli* cells [32]. The principle of regulatory FBA is illustrated with the example network in Figure 3A.

The flux balance view can thus account for some instances of diauxic growth, but its explanatory value is limited because the switching between the use of alternative metabolic pathways crucially depends on measured maximal fluxes and auxiliary capacity constraints [33]. Where do these constraints come from and how do they fit into the global picture of the supposed optimal adaptation of microbial metabolism to its environment? One way to answer this question is to consider the optimization problem from the perspective of resource allocation.

Resource allocation view

In which activities should a business company invest to maximize its profits? Cells can be seen as facing similar questions of resource allocation. They are self-reproducing systems generating energy and precursors from nutrients in their environment, to produce macromolecular structures including ribosomes and enzymes that are necessary for the synthesis of daughter cells. The limited resources extracted from the nutrients, as well as physical resources such as cell volume and membrane space, need to be distributed over a range of cellular processes. If the cell is to optimize its growth rate it must make trade-offs between conflicting demands for these resources. Can the resource allocation perspective explain diauxic growth?

The flux balance view described in the previous section has been extended to a (static or dynamic) optimization problem with additional constraints on the available resources. One obvious constraint is the existence of an upper bound on the total enzyme concentration of the cell, given by physical and physiological constraints on cell volume, cell density, and the enzymatic fraction of cell mass [34]. When combined with high-throughput proteomics measurements [35] or prior information on enzyme molecular weights and catalytic constants [11,36,37], this yields an additional global constraint on fluxes through the
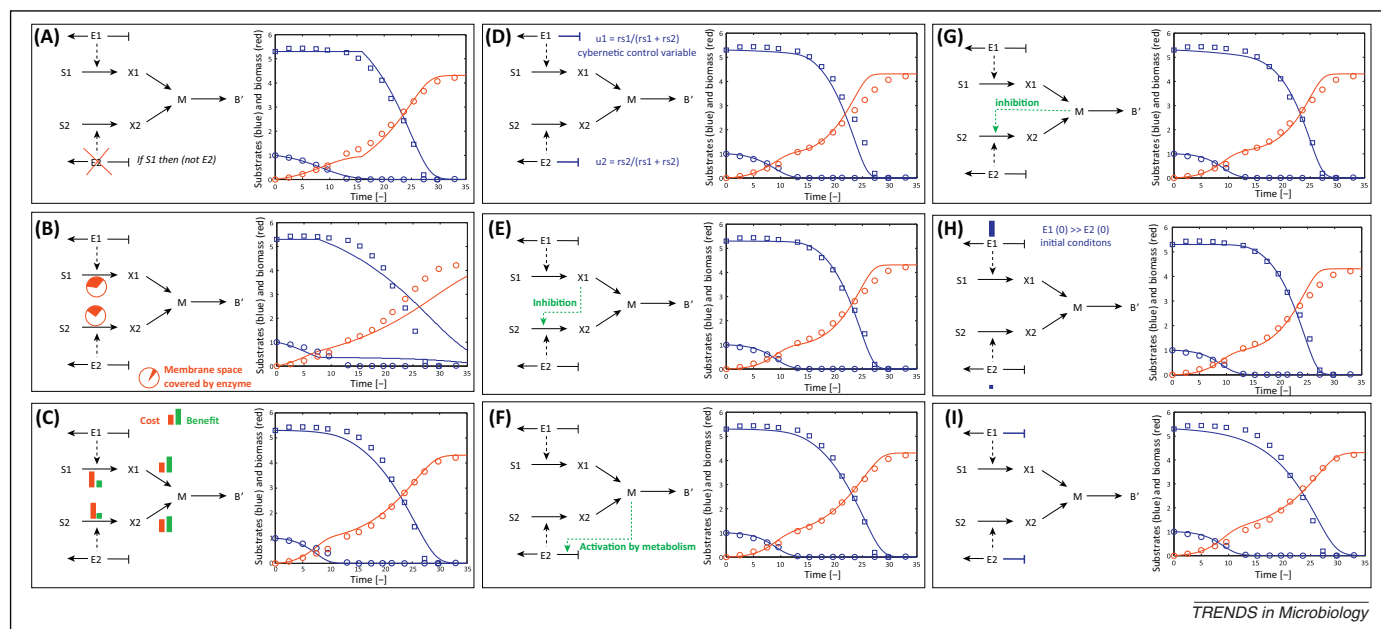
Figure 3. Different explanations of diauxic growth: models and their time-course predictions, compared with experimental data [17]. Glucose, blue circles; lactose, blue squares; biomass, red circles). **(A)** Regulatory flux balance analysis. The rule exemplifies the (in)activation of enzymes in response to environmental conditions. **(B)** Resource allocation. The pie charts represent the proportion of resources (here membrane space) allocated to each reaction. **(C)** Cost–benefit trade-off. The bar graphs represent costs (red) and benefits (green) for each reaction, that is, the energy required for the synthesis of the enzyme or transporter, and the energy generated by the flux through the reaction, respectively. **(D)** Cybernetic approach. The cybernetic variables represent the fractional allocation of resources to the synthesis of the enzymes. **(E)** Inducer exclusion. **(F)** Transcription activation. **(G)** Hypothetical inhibition of inducer synthesis by a central metabolite. **(H)** Enzyme induction, but with different levels for the initial values. **(I)** Enzyme induction with different values for maximal rate of enzyme synthesis. S_1, S_2, substrates; X_1, X_2, M, metabolites; E_1, E_2, enzymes; B', internal biomass compartment; u_1, u_2, cybernetic control variables, $rs1$, $rs2$, fluxes through reactions consuming S_1, S_2, respectively.

network. The use of this constraint leads to a variant of FBA, termed FBA with molecular crowding (FBAwMC), which has been used to predict the sequence and the mode of substrate uptake in wild type and mutant *E. coli* cells. In particular, the model was capable of reproducing the temporal order of substrate utilization in a batch culture containing five different carbon sources [11]. Another constraint can be formulated by taking into account the competition for limited membrane space between nutrient transporters and respiratory chain proteins, and this was found to improve predictions on the relative utilization of respiration and fermentation in growing *E. coli* cells [13].

Figure 3B illustrates how an additional constraint on the total enzyme abundance in an optimization framework can reproduce diauxic growth. The example is reminiscent of the so-called cybernetic models developed by Ramakrishna and colleagues [38,39]. Cybernetic models provide a coarse-grained description of microbial kinetics and allocate resources to the synthesis or the activity of specific enzymes in proportion to their return, that is, the growth rate on the substrate metabolized by the enzyme. The different variants of cybernetic modeling have been capable of accounting for a variety of scenarios of simultaneous or preferential uptake of carbon sources in *E. coli* [38,40].

The application of resource allocation approaches usually requires an estimate of the total mass (or concentration) of enzymes available in a given growth condition. Hwa and colleagues have analyzed the proteome of *E. coli* in more detail and developed growth laws to phenomenologically describe how the distribution of total protein over sectors of the proteome, each representing a class of proteins with a specific function, varies with the growth rate

[41,42]. The model predicts that, with increasing growth rate, the fraction of protein that is involved in catabolism decreases, while the fraction of protein involved in anabolism increases [42]. Moreover, as is well documented, the fraction of protein that is involved in transcription and translation also increases with the growth rate. The latter observation highlights that the abundance of the gene expression machinery is adjusted across growth conditions, and cannot simply be considered constant (as is done in many models). Several recent studies have suggested that the latter effect may be important for the expression control of particular genes [43–47], including genes that encode global transcription regulators and enzymes involved in carbon metabolism, such as Crp [43].

It is important to note that flux constraints originating from resource limitations to a large extent root in metabolism itself, which produces the energy and precursors necessary for the synthesis of enzymes [48]. This autocatalytic nature of metabolism has been captured in several recent models that describe the fueling of gene expression by metabolism and the control of metabolic fluxes by the products of gene expression. For example, FBA models that complete the genome-wide reconstruction of *E. coli* metabolism have been extended with reactions involved in the synthesis of more than 1000 *E. coli* proteins [12]. Optimization of the growth rate in the resulting huge model has been shown to lead to more accurate predictions of growth rate and substrate uptake/byproduct secretion rates across several different growth conditions, as well as generating testable predictions about protein levels (see also [33,49]).

Resource allocation is fundamentally an optimization problem, focusing on supposedly optimal flux distributions

without worrying about the actual regulatory mechanisms the cell has developed to control fluxes. However, the results of the approach can be used to understand the advantage conferred by the existence of particular regulatory mechanisms. This point has notably been developed in the context of the transcriptional regulation of metabolic enzymes [14–16,50,51]. Specifically, the cell has to weigh the growth benefit provided by a regulatory mechanism against the cost or metabolic burden it incurs. One would expect that the investment in the synthesis of a regulatory protein controlling a metabolic operon will only pay off if the expression of the operon is costly and the need for the resulting enzymes strongly varies between conditions. Growth on two carbohydrates has not been considered from this cost–benefit perspective. However, as illustrated for the example network in Figure 3C, it can in principle reproduce diauxic growth.

The resource allocation perspective is capable of successfully predicting, in a variety of diauxic growth conditions, the substrate uptake and assimilation patterns of bacteria and the growth rates they sustain. Its value lies in uncovering a rationale for the observed behavior by relating it to an optimality criterion. However, because it largely ignores the regulatory mechanisms achieving resource allocation, it cannot provide a causal explanation of the phenomena.

Mechanistic view

Among the regulatory mechanisms that have been shown to play a role in diauxic growth, enzyme induction was the first to be discovered [52]. It involves the repression of the genes required for the assimilation of a substrate by a transcription factor, as well as the release of this repression by an inducer molecule when the substrate is available in the growth medium (Figure 2). Well-known examples of enzyme induction in *E. coli* are the regulation of the lactose and arabinose operons [53,54]. In addition to operon-specific mechanisms, there are global regulators of carbohydrate uptake and metabolism [2,55]. In *E. coli* the major global player is the transcription activator Crp (cAMP receptor protein) [56,57]. Crp is encoded by the gene *crp* and influences positively and/or negatively more than 260 operons [58]. Crp is activated by cAMP, a second messenger that is synthesized by the adenylate cyclase Cya. cAMP synthesis is classically believed to be controlled by the concentration of Cya on the one hand and the phosphorylation state of the phosphoenolpyruvate-carbohydrate phosphotransferase (PTS) system on the other. At high glucose-uptake rates, the glucose-specific IIA component of the PTS, EIIA^{Glc}, is mostly unphosphorylated but, when glucose is depleted from the medium, the phosphorylated form becomes dominant and activates Cya [1,59]. Recent findings, however, suggest that other metabolites, such as α-ketoglutarate, oxaloacetate, and also pyruvate might act as effectors of Cya too [42]. The PTS is also involved in another regulatory mechanism of CCR, inducer exclusion. In the presence of glucose, unphosphorylated EIIA^{Glc} inhibits the activity of several non-PTS permeases, as well as glycerol kinase, to inhibit the uptake and metabolism of alternative carbon sources [1,60].

To what extent can the above regulatory mechanisms quantitatively account for diauxic growth phenomena? Given the multilayered complexity of the regulatory networks schematized in Figure 2, this question is difficult to answer by intuitive reasoning alone. This has motivated the development of quantitative ordinary differential equation (ODE) models, which integrate knowledge on the molecular mechanisms and the parameters describing the kinetics of these mechanisms. The stoichiometry of the underlying biochemical reactions also provides the starting-point for these models but, unlike the FBA approaches discussed above, explicit expressions for the reaction rates are provided (Box 1). The added descriptive power of the model comes at a price – namely that quantitative values for all kinetic parameters need to be provided, either by direct biochemical measurements or by estimation from steady-state or time-course data, typically concentrations of enzymes and metabolites [61–63]. Because direct measurements of biochemical parameters may not be available or unreliable, unless special care is taken to approach *in vivo* conditions [64] – and parameter estimation in complex nonlinear mathematical models is a difficult problem [65–67] – this step remains the most important bottleneck in the development of mechanistic models.

Mechanistic models of CCR in bacteria have a long history, with early examples dating back to the 1970s and 1980s [68,69]. Over the past decade, increasingly more-complex models have been developed taking into account one or several of the above-mentioned regulatory mechanisms [17,18,70]. Table 1 summarizes and compares a few well-known examples of models of central carbon metabolism in *E. coli* and its regulation. Some models additionally account for spatial effects, for example diffusion limitations in PTS functioning [71], while others integrate the inherent stochasticity of gene expression [72,73].

The model of Bettenbrock and colleagues describes the expression of 17 enzymes in *E. coli* central carbon metabolism, 38 enzymatic reactions, and the dynamic behavior of more than 50 metabolites, accounting for enzyme induction, cAMP regulation, and inducer exclusion [17]. While many of the parameters could be obtained from the experimental literature, about one third needed to be estimated from dedicated time-series experiments in which growth rate, external metabolites, and gene expression were measured. Among other aspects, the model was able to faithfully reproduce glucose–lactose diauxie in several genetic backgrounds and growth conditions. It confirmed the known role of inducer exclusion in preventing lactose uptake in the presence of glucose, but also demonstrated the importance of the occurrence of a cAMP pulse during the transition period for the adaptation of cellular physiology. In particular, the transcriptional regulation of the *pts* genes by Crp·cAMP, while often neglected, was found to be quantitatively important, a prediction that would have been difficult to make without the model.

The model of Kotte and colleagues has taken this work further by coupling transcriptional regulation of enzyme-encoding genes to intermediates of central carbon metabolism [18]. The analysis of the model has led to the insight that changes in metabolic fluxes, for example during diauxic

Table 1. Comparison of models for CCR[a,b]

	Wong [69]	Kremling [92]	Degenring [93]	Sauter [94]	Lee [95]
Type of equation	DA	DA	ODE	ODE	Algebraic, logic rules
Kinetics	Yes	Yes	Yes	Yes	No (constraints)
# State variables	13	20	10	22	Not determinable
# Kinetic parameters	65	90	127	53	
Network modules					
- Uptake systems	Glc, Lac	Glc, Lac	Glc	Scr	Glc, Lac
- Glycolysis		×	×	×	
- TCA					
- PPP			×		
- Global regulation	cAMP·Crp	cAMP·Crp		cAMP·Crp	21 regulatory proteins
- Specific regulation	LacI	LacI			(e.g., Crp, LacI, GalR)
- Allosteric control	No	Yes[c]		Yes[c]	No
Experimental validation	Parametric sensitivity	Literature, selected state variables measured (wild type, mutant strains)	Parametric sensitivity	Pulse experiment, stop feeding	Qualitative batch experiment

	Bettenbrock [17]	Asenjo [96]	Nishio [97]	Covert [98]	Baldazzi [99]
Type of equation	DA	Discrete, logic rules	DA	DA, logic rules	DA
Kinetics	Yes	Stochastic	Yes	Partly	No
# State variables	67	67	63	282	40
# Kinetic parameters	320		131	8	
Network modules					
- Uptake systems	Glc, Lac, Gly, Glc6P, Ac	Glc, Gly, Ac	Glc	11 carbohydrates	Glc
- Glycolysis	×	×		×	×
- TCA		×		×	
- PPP				×	
- Global regulation	cAMP·Crp	cAMP·Crp, FruR	cAMP·Crp	16 transcription factors	cAMP·Crp, FruR, Fis, RpoS
- Specific regulation	LacI, GalS, GalR, Mlc	Mlc	Mlc		
- Allosteric control	Yes[c]	No	No	Yes (for dynamic part)	Yes[c]
Experimental validation	Selected state variables, 18 experiments (wild type, mutant strains)	Literature, batch experiments	Steady-state data for mutant strains	Single gene perturbation from literature, batch experiments	No

	Kotte [18]	Berthoumieux [65]	Peskov [70]	Matsuoka [100]	
Type of equation	DA	DA	ODE	DA	
Kinetics given	Yes	No	Yes	Yes	
# State variables	47	23	48	35	
# Kinetic parameters	193	Yes	476	99	
Network modules					
- Uptake systems	Glc, Ace	Glc	Glc	Glc	
- Glycolysis	×	×	×	×	
- TCA	×	×	×	×	
- PPP	×	×	×	×	
- Global regulation	cAMP·Crp, FruR			cAMP·Crp, FruR	
- Specific regulation	PdhR, IclR			PdhR, IclR	
- Allosteric control	Yes[c]	Yes[c]	Yes[c]	Yes[c]	
Experimental validation	Complete set for all state variables (steady-state)	Literature	Steady-state dataset	Batch experiments with wild type and mutant strains	

[a]Models are named according to the first author of the publication.

[b]Abbreviations: Ac, acetate; DA, differential algebraic system; Glc, glucose; Glc6P, glucose 6-phosphate; Gly, glycerol; Lac, lactose; ODE, ordinary differential equation system; PPP, pentose phosphate pathway; Scr, sucrose; TCA, tricarboxylic acid cycle. The symbol × indicates that the respective subnetwork is considered in the publication; #, number.

[c]For details see original publications.

growth, are captured in a distributed way at different locations in the metabolic network. The information provided by these sensors, notably Crp·cAMP, Cra (catabolite repressor activator)·FBP (fructose 1,6-biphosphate), IclR (isocitrate lyase regulator)·GLX (glyoxylate)·PYR (pyruvate), and PdhR (pyruvate dehydrogenase complex regulator)·PYR, feed back into the regulation of the fluxes, either in the short run (by modulating enzyme activity) or in the long run (by affecting gene expression). Recently, a follow-up study provided further experimental evidence for Cra·FBP being a sensor of glycolytic flux [74]. While these and other studies have succeeded in integrating known interactions into a single model, there remains uncertainty about, for example, the molecular details of EIIA^{Glc} activation of Cya [2,75] and FBP inhibition of Cra [76,77].

Kinetic models of the example network in Figure 1, accounting for enzyme induction, global gene expression regulation, and inducer exclusion, allow a good fit to the glucose–lactose data for chosen parameter values (Figure 3E–G). The model accounting for enzyme induction only (Figure 3H,I) corresponds to a minimal model for CCR proposed by Narang and colleagues [19]. This minimal model is able to predict diauxic growth, and also explains the general observation that the preferred substrate supports faster growth than the less-preferred substrate [78]. A key element of this model, in addition to enzyme induction, is the passive control of enzyme concentrations by the growth rate. Fast growth on a preferred substrate results in strong dilution of the enzymes, in particular the non-induced enzymes for the less-preferred substrate, thus preventing utilization of the latter. The minimal model also accounts for the observation that, at low growth rates, alternative substrates can be utilized simultaneously, as observed experimentally [79]. While providing an elegant explanation for diauxic growth, it should be noted that growth on some substrates, such as acetate, requires global transcriptional regulation by cAMP. A strain with a *cya* deletion cannot express the enzyme acetyl-CoA synthetase (Acs), a key enzyme in acetate metabolism [80], and has a severe growth defect.

While current mechanistic models can thus explain some instances of diauxic growth, they are usually limited to a restricted range of phenomena. One reason may be the existence of regulatory connections that are unknown or unaccounted for, such as α-ketoacids coupling carbon catabolic fluxes to nitrogen and sulfur availability [42,81,82]. More generally, active or passive mechanisms bringing about the resource allocation constraints discussed in the previous section may play a role. Thus far, no mechanistic model has integrated effects such as the competition for limited membrane space and changes in the activity of the transcriptional and translational machinery as well as mRNA stability [83] during growth transitions.

Concluding remarks
Microbial systems biology aims at gaining a systems-level understanding of the functioning of microbial cells, using a combination of mathematical modeling and experiments [7,84,85]. We have illustrated this by revisiting a classical phenomenon in microbiology, CCR and diauxic growth in

Escherichia coli, from a systems-biology perspective. We have notably considered models that view diauxic growth as arising from the maximization of growth rate in a network of metabolic reactions, the optimal allocation of scarce resources to the synthesis and activity of enzymes involved in different metabolic pathways, and the operation of interlocking regulatory mechanisms at the molecular level. Probably the most interesting novel insight collectively emerging from all these models is that the different approaches are able to quantitatively reproduce diauxic growth with some success and, moreover, can explain specific aspects of its functioning that would have been difficult to achieve otherwise. However, each approach comes with limitations owing to its particular way of framing the problem, thus restricting its range of applicability and its appropriateness for answering specific biological questions.

The application of flux balance models depends on the definition of capacity constraints and usually requires additional regulatory constraints for enabling or disabling specific metabolic pathways depending on the carbon sources present in the growth medium. While the use of FBA for explaining diauxic growth is thus limited, extensions that take into account global resource allocation constraints are more powerful. They define an extended optimization problem from which the capacity and regulatory constraints in FBA naturally arise. However, the major strength of these approaches – the ability to make quantitative predictions from minimal information on regulatory mechanisms using an optimality criterion – is also their major weakness. The choice of an optimality criterion is obviously crucial, but controversial [12,20–22]. While most studies assume that the cell optimizes its growth rate, there are examples of bacteria that exhibit a so-called inverse diauxie, preferring carbon sources that sustain a lower growth rate [86]. It is also not clear to which extent the models preserve their predictive capability in situations where optimality arguments may not apply, for example in mutant strains. More fundamentally, resource allocation models may be helpful in recognizing why diauxic growth occurs, but will not be of much use in understanding how the cell has implemented sequential uptake. Mechanistic models of central carbon metabolism do capture many of the known molecular mechanisms, and thus have the potential of providing such explanations. A major disadvantage of mechanistic models, however, is that they are difficult to calibrate. Moreover, they often do not generalize beyond the specific experimental scenarios for which they have been designed because they focus on a small module of the regulatory network, ignoring the interactions of this module with the rest of the cell. In particular, they usually do not account for global effects due to physical constraints (molecular crowding, membrane occupancy, growth-rate dilution) and due to mechanisms controlling global physiological effects (activity of the transcription and translation machinery, mRNA and protein stability).

All the proposed models in the literature probably capture important aspects of diauxic growth and CCR: bacterial metabolism in flux balance models, global physical and physiological constraints in resource allocation

Box 2. Outstanding questions

- What is the relative importance of the different regulatory mechanisms that have been demonstrated to play a role in CCR?
- How can the supposed optimal functioning of bacterial metabolism be related to the known regulatory mechanisms of CCR?
- How can global cell physiology be integrated into existing mechanistic models of CCR?
- How can the resulting multiscale dynamic models be reliably calibrated from experimental data?

models, and specific regulatory mechanisms in mechanistic models. We believe that a new generation of models is needed, including all these aspects. Such models would be precious for addressing open questions in CCR, for example the precise role of the central signaling molecule cAMP. The common textbook explanation of CCR assigns a major role to cAMP, allowing it to activate operons of less preferred carbon sources like lactose, when preferred carbon sources such as glucose have been depleted [56,57]. This explanation is notably based on the correlation between glycolytic fluxes, the phosphorylation state of the PTS, and the activity of Cya ([1] and references therein). However, it is in conflict with the observation that the concentration of cAMP is almost identical when *E. coli* cells are growing on either glucose or lactose [87,88]. Moreover, there is evidence that inducer exclusion is mostly responsible for glucose–lactose diauxie [19,87]. Recent work has suggested a completely different role for cAMP, namely coordinating the expression of catabolic proteins with biosynthetic and ribosomal proteins, in accordance with the metabolic needs of the cell [42]. Outstanding questions are listed in Box 2.

A variety of other interesting questions can be mentioned that could be profitably addressed by these models, including a comparison of the variety of molecular implementations of CCR in different organisms and their functional properties, and the analysis of more complex ecological growth scenarios. This requires models that view the cell as a whole, instead of focusing on specific metabolic and regulatory networks in isolation from the cellular environment. In recent years, this whole cell perspective has gained ground, with the model of *Mycoplasma genitalium* as a landmark achievement [89]. It provides a detailed description of all known molecular components of the cell and the biochemical reactions and regulatory interactions in which they are involved. Nevertheless, it is not always necessary to develop a whole cell perspective at a detailed molecular level. As illustrated in this review, even extremely simplified models may explain aspects of a complex phenomenon such as diauxic growth, and can capture the essential dynamic features of the mechanisms at work. For many questions, a global view of the functioning of the cell may be obtained using coarse-grained models [33,90], providing the cellular context missing in mechanistic models of specific cellular processes.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tim.2014.11.002.

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