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Importance of Metabolic Coupling for the Dynamics of Gene Expression Following a Diauxic Shift in *E. coli* ⋆

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Abstract: Gene regulatory networks consist of direct interactions on the transcriptional level, but also include indirect interactions mediated by metabolism. We investigate to which extent these indirect interactions influence the dynamics of the system. To this end, we build a qualitative model of the gene regulatory network controlling carbon assimilation in *E. coli*, and use this model to study the changes in gene expression following a diauxic shift from glucose to acetate. We find significant differences between the dynamics of the system in the absence and presence of metabolic coupling. This shows that interactions arising from metabolic coupling cannot be ignored when studying the dynamics of gene regulatory networks.

Keywords: Bioinformatics, systems biology, modeling and identification, microbiology, hybrid models, gene regulatory networks

1. INTRODUCTION

The reorganization of gene expression patterns in response to environmental changes is controlled by so-called gene regulatory networks, which ensure the coordinated expression of clusters of functionally related genes. The interactions in the network may be direct, as in the case of a gene coding for a transcription factor regulating the expression of another gene. Most of the time, however, regulatory interactions are indirect, *e.g.* when a gene encodes an enzyme producing a transcriptional effector (Brazhnik et al., 2002). The latter interactions arise from the fact that gene regulation is embedded in a complex, multi-level system that tightly integrates gene expression with metabolism. We call the occurrence of indirect interactions between enzymes and genes, mediated by metabolism, *metabolic coupling*.

In previous work, we showed how indirect interactions arising from metabolic coupling can be derived from a model of the underlying biochemical reaction network (Baldazzi et al., 2010). We applied this approach to the carbon assimilation network in *Escherichia coli*. Our results showed that the derived gene regulatory network is densely connected, contrary to what is usually assumed. Moreover, we found that the signs of the indirect interactions are largely fixed by the direction of metabolic fluxes, independently of specific parameter values and rate laws, and that a change in flux direction may invert the sign of indirect interactions. This leads to a feedback structure that is at the same time robust to changes in the kinetic properties of enzymes and that has the flexibility to accommodate radical changes in the environment.

It remains an open question, however, to which extent the indirect interactions induced by metabolic coupling influence the dynamics of the system. This is a key issue for understanding the relative contributions of the regulation of gene expression and metabolism during the adaptation of the cell to changes in its environment. Indirect interactions could be essential in shaping the response of the cell, giving it the required flexibility to adapt to external perturbations. However, one could also argue that indirect interactions only have a fine-tuning effect, simply refining the gene expression levels at which the system would stabilize without metabolic coupling.

In order to decide between these two hypotheses, and obtain a clearer view of the role of metabolic coupling in the adaptation of gene expression, we build a dynamic model of the gene regulatory network controlling carbon assimilation in *E. coli*, and use this model to study the changes in gene expression following a diauxic shift from glucose to acetate. More specifically, we develop a qualitative model using piecewise-linear (PL) differential equations that allows us to encode the regulatory logic of the system in a simple way (Batt et al., 2008; Glass and Kauffman, 1973). Even though good quantitative models of carbon

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metabolism in *E. coli* have appeared in recent years (*e.g.*, Bettenbrock et al. (2005); Kotte et al. (2010)), not much is know about precise mechanisms and parameters values for gene regulation. Moreover, qualitative models are an appropriate tool for analyzing if metabolic coupling can induce *major* changes in the gene expression dynamics, *i.e.*, will not only have an effect on quantitative but also qualitative properties of the system dynamics.

We build two distinct qualitative models of the network, corresponding to the topology with and without indirect interactions, respectively. The dynamical properties of the two models are analyzed and compared with available experimental data. In particular, we compare the steady-state concentration of enzymes and transcription regulators during growth on glucose and acetate, as well as the dynamic response of gene expression to the exhaustion of glucose and the subsequent assimilation of acetate.

We find significant differences between the dynamics of the system in the absence and presence of metabolic coupling. This underlines the importance of metabolic coupling in gene regulatory networks, and show that such indirect interaction cannot be neglected when studying the adaptation of an organism to changes in its environment.

2. METABOLIC COUPLING IN GENE REGULATORY NETWORKS

In Baldazzi et al. (2010) a mathematical method is proposed, based on a combination of time-scale approximations and sensitivity criteria from metabolic control analysis, to reconstruct the indirect interactions between genes from a model of the underlying network of biochemical reactions. In the following, we briefly summarize the basic principles of this approach.

We start by building a stoichiometric model of the network. The model takes the form of a system of ordinary differential equations (ODEs) and describes the rate of change of the concentrations of the different molecular species in the network:

$$\dot{x} = N\, v(x), \qquad x(0) = x_0, \tag{1}$$

where $x \in \mathbb{R}^n_+$ denotes the vector of concentrations and $v : \mathbb{R}^n_+ \to \mathbb{R}^q$ the vector of reaction rates. $N \in \mathbb{Z}^{n\times q}$ is a stoichiometry matrix. In the presence of conserved quantities, N is the reduced stoichiometry matrix.Contrary to kinetic models, the rate laws are not explicitly specified, but only the functional dependency of the reaction rates on specific molecular species are given. We do not develop the rate laws, because only the signs of the partial derivatives are used for reconstructing the indirect interactions (see below).

As a first step, the model can be simplified by making the QSS approximation (Heinrich and Schuster, 1996). Two different time-scales are distinguished, one corresponding to the slow processes (protein synthesis and degradation) and one to the fast processes (complex formation and enzymatic reactions). Based on time-scale separation, the original model can be rewritten into two distinct subsystems

$$\dot{x}^s = N^s\, v^s(x^s, x^f), \qquad x^s(0) = x^s_0, \tag{2}$$

$$\dot{x}^f = N^f\, v^f(x^s, x^f), \qquad x^f(0) = x^f_0 \tag{3}$$

where $x^s \in \mathbb{R}^m_+$ and $x^f \in \mathbb{R}^{n-m}_+$ are vectors of slow and fast variables, respectively, and N^s, N^f and v^s, v^f the corresponding stoichiometry matrices and rate vectors. The slow variables describe total concentrations of the proteins, and the fast variables concentrations of metabolites and complexes of proteins and signalling molecules.

The QSS approximation makes the assumption that at the time-scale of the slow processes the fast part of the system is at steady state, instantly adapting to the dynamics of the slow variables, *i.e.* $N^f\, v^f(x^s, x^f) = 0$ (see Heinrich and Schuster (1996) and Khalil (2001) for the conditions under which the QSS approximation is valid). This means that, after an initial transient, the dynamics of the fast system can be well approximated by an algebraic function of the slow variables, if such a function can be found: $x^f = g(x^s)$, $g : \mathbb{R}^m_+ \to \mathbb{R}^{n-m}_+$. The resulting reduced system, at the slow time-scale, takes the following form

$$\dot{x}^s = N^s\, v^s(x^s, g(x^s)) \tag{4}$$

The interest of this model is that it represents the structure of direct and indirect interactions between the slow variables, that is, the structure of the gene regulatory network. Since we have not specified the rate laws, and in general these are complex nonlinear functions of the concentration variables, it is not possible to obtain a closed-form expression for the function g. We therefore follow another strategy to characterize the indirect interactions between the slow variables, by studying the Jacobian matrix $\mathcal{J} \in \mathbb{R}^m \times \mathbb{R}^m$ of the system in Eq. 4:

$$\mathcal{J} = \frac{\partial \dot{x}^s}{\partial x^s} = N^s\, \frac{\partial v^s(x^s, g(x^s))}{\partial x^s} + N^s\, \frac{\partial v^s(x^s, g(x^s))}{\partial x^f}\, \frac{\partial g(x^s)}{\partial x^s} \tag{5}$$

The Jacobian matrix includes the direct effect of each slow variable on the others (first term) and the indirect effect via coupling through the fast system (second term). It accounts for direct regulation of gene expression by transcription factors as well as indirect regulation through metabolic intermediates. Applying the implicit function theorem to the QSS equation, $\partial g(x^s)/\partial x^s$ can be further developed as

$$\frac{\partial g(x^s)}{\partial x^s} = -M^{-1}\, N^f\, \frac{\partial v^f(x^s, x^f)}{\partial x^s}. \tag{6}$$

where $M = N^f\, \partial v^f(x^f, x^s)/\partial x^f$ is the Jacobian matrix of the fast system. Eq. 6 describes the response of the fast system around its steady state to changes in the slow variables. $-M^{-1}\, N^f$ is known as the matrix of (non-normalized) concentration control coefficients in metabolic control analysis (Heinrich and Schuster, 1996).

The symbolic computation of the elements of $\mathcal{J}$ gives rise to complex expressions in terms of elementary partial derivatives of the reaction rates with respect to the concentration variables, called (non-normalized) elasticities (Heinrich and Schuster, 1996). Even in the absence of a precise specification of kinetic rate laws and parameters values, most elasticities have a well-defined sign, given

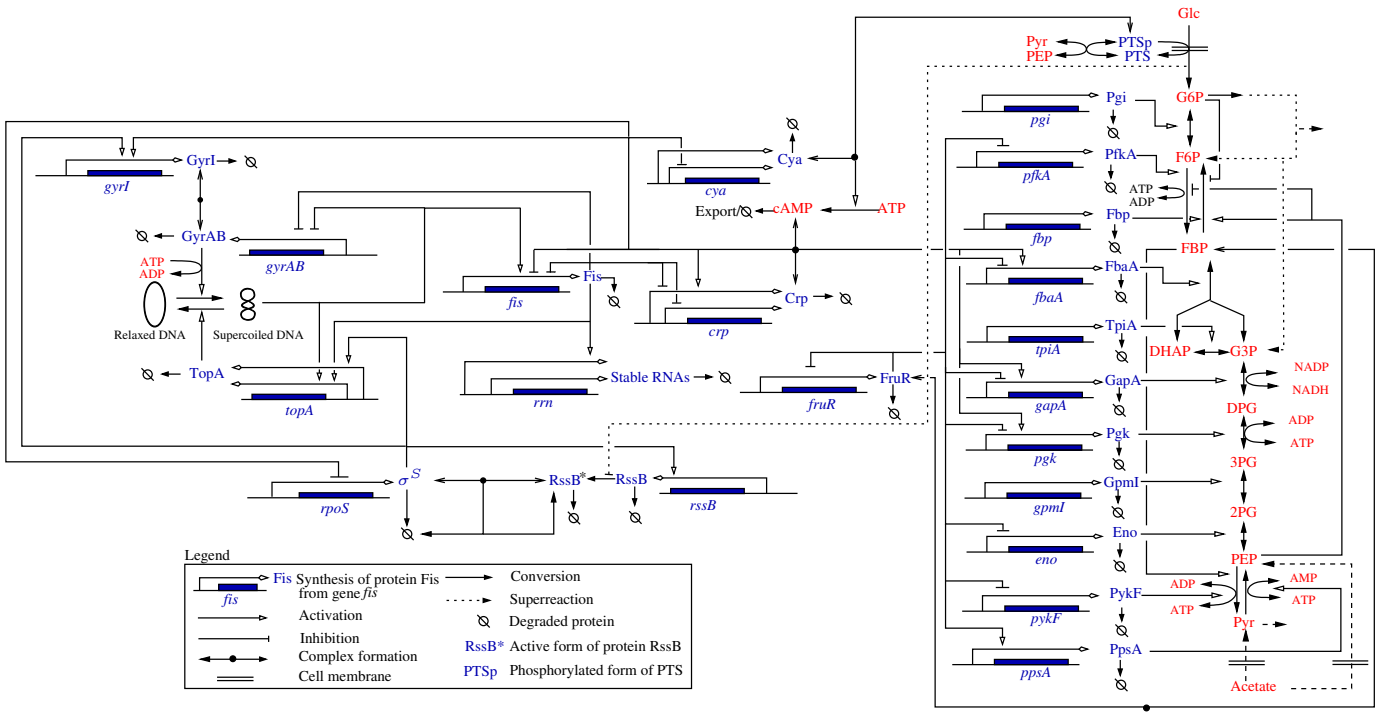

Fig. 1. Carbon assimilation network in *E. coli*, consisting of the glycolysis and gluconeogenesis pathways and their genetic and metabolic regulation (Baldazzi et al., 2010).

a convention on the positive flux direction (Gutierrez-Ríos et al., 2007). Together with constraints imposed by the stability of the steady state of the fast system, the elasticities are used to evaluate the signs of the elements of the Jacobian matrix. Notice that the sign of the elasticity of reversible reactions with respect to enzyme concentrations can be positive or negative, depending on the flux direction. As a consequence, the signs of the elements of $\mathcal{J}$ depend on the growth conditions, as the latter may influence the flux directions.

3. RESULTS AND DISCUSSION

Fig. 1 shows a part of the network involved in the assimilation of carbon sources in *E. coli*. It consists of the glycolytic and gluconeogenic pathways, the phosphotransferase system involved in glucose uptake, the genes coding for the enzymes, their key transcriptional regulators (Crp, FruR, and Fis), as well as other global regulators of transcription (RpoS, DNA supercoiling, ...). We distinguish two different growth conditions: growth on the carbon-rich substrate glucose (glycolysis) and growth on the carbon-poor substrate acetate (gluconeogenesis). Since glucose and acetate are utilized by distinct metabolic pathways, the flux distributions during glycolysis and gluconeogenesis are significantly different. A diauxic shift from glucose to acetate notably leads to the inversion of glycolytic fluxes (Oh et al., 2002).

The network of biochemical reactions in Fig. 1 can be transformed into a network of direct and indirect regulatory interactions following the method outlined in Sec. 2. To this end, we use a stoichiometric model of the network describing the dependence of the reaction rates on the substrates, products, enzymes, transcription factors, and other molecular species (Baldazzi et al., 2010). In the case of reversible reactions, the signs of the elasticities, and therefore the signs of the indirect interactions, depend on the directions of the metabolic fluxes. We therefore consider the cases of glycolysis and gluconeogenesis separately.

Fig. 2 shows the resulting networks for glycolysis (panel A) and gluconeogenesis (panel B). The boxes represent so-called coupling species, which channel indirect influences on gene expression through metabolism. Some indirect interactions appear in one growth condition and disappear in the other, such as the regulatory influence of GapA on Crp·cAMP and its targets. Moreover, the same interaction may have an opposite sign in the two cases, for instance the effect of GapA on the concentration of free FruR. Metabolic coupling thus allows the structure of regulatory interactions to be dynamically rewired by changes in the environment (Baldazzi et al., 2010).

The networks in Fig. 2 have been simplified by leaving out some of the glycolytic enzymes. We notably omit enzymes whose expression does not significantly change between growth on glucose and acetate (Oh et al., 2002), enzymes that are constitutively expressed, and enzymes that do not regulate any other gene in the network. Moreover, we keep only a single representative of each group of enzymes with common regulators. This leaves us with GapA, PpsA, and PykF, whose expression patterns are known to characterize the two modes of carbon catabolism considered here (Oh et al., 2002). Notice that these simplifications of the network tend to reduce the number of indirect interactions, and thus their influence on the network dynamics. If an effect of the indirect interactions on the qualitative dynamics is visible in the simplified networks, this will *a fortiori* be the case in the extended networks as well.

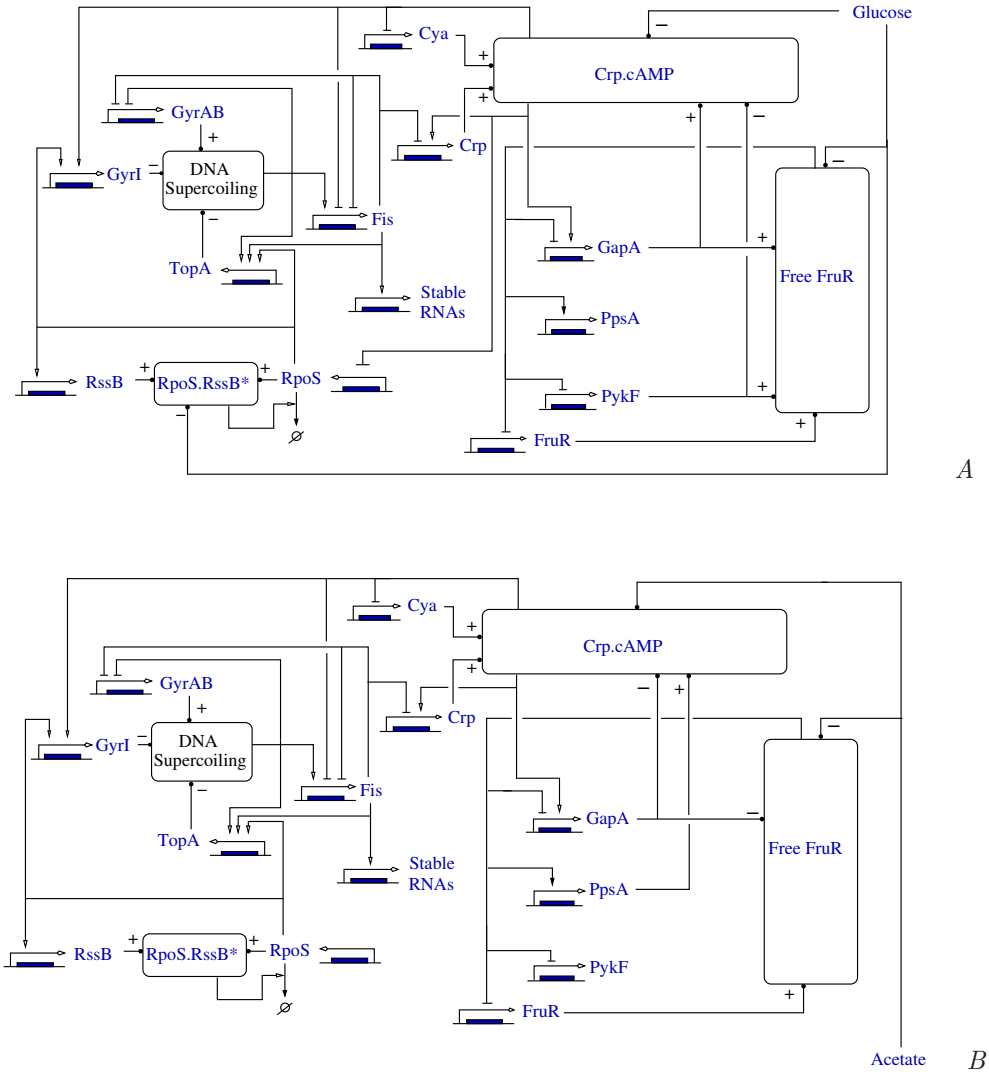

Fig. 2. Gene regulatory networks for the glycolytic (A) and gluconeogenic case (B), obtained from the network of biochemical reactions shown in Fig. 1. The networks are denoted by $\mathcal{M}_{glyco}$ and $\mathcal{M}_{neo}$, respectively. The boxes indicate the coupling species, here Crp·cAMP, free FruR, RpoS·RssB, and DNA supercoiling.

In order to precisely assess the effect of the indirect interactions on the network dynamics, we additionally define a reference network consisting of transcriptional regulatory interactions only. This network is called $\mathcal{M}^0$, and is the same for glycolysis and gluconeogenesis.

The networks shown in Fig. 2 are gene regulatory networks, in the sense that they describe the (direct or indirect) influence of the products of one gene on the expression of another gene (Bolouri, 2008). The interactions can be positive (activation) or negative (inhibition). A variety of methods exist to model and analyze the dynamics of gene regulatory networks (Karlebach and Shamir, 2008). Here we focus on the use of qualitative models, which provide a coarse-grained description of the dynamics of gene expression, in the sense that they include the logic of gene regulation without explicitly specifying the biochemical mechanisms.

More specifically, we use so-called *piecewise-linear (PL) differential equations*, a formalism originally introduced by Glass and Kauffman (1973) for describing gene regulatory networks. The PL models have favorable mathematical properties that allow their qualitative dynamics to be analyzed, even in higher-dimensional systems (Kappler et al., 2003). In particular, the dynamics of the system can be represented by means of a state transition graph, which can be inferred from the model using orderings between parameter values rather than exact numerical values.

The network structures in Fig. 2 are transformed into qualitative PL models by defining the differential equations for each of the gene products (protein concentrations) as well as the ordering of the parameters that characterizes the system dynamics (Batt et al., 2008). Intuitively, this amounts to specifying the logic of the regulation of the genes and the relative strength of the different interactions regulating the expression of a gene. The PL models for the $\mathcal{M}_{glyco}$ and $\mathcal{M}_{neo}$ networks extend an existing qualitative model of the network of global regulators in *E. coli* (Ropers et al., 2006), by adding metabolic coupling induced by carbon catabolism. The following principles have guided the modeling of the network:

- The existence of a single stable attractor in each growth condition, and the absence of glycolytic oscillations in the normal range of operation;
- A parametrization of the models consistent with that of the previous models;
- No zero expression of glycolytic enzymes during growth on glucose, no zero expression of gluconeogenic enzymes during growth on acetate.

The $\mathcal{M}^0$ model is obtained from the $\mathcal{M}_{glyco}$ model by eliminating all regulators that are not transcription factors. The models are given in Baldazzi et al. (2011), together with an analysis of the robustness of our results to changes in parameter constraints.

The steady-state concentrations of the enzymes and regulatory proteins involved in carbon assimilation, both during growth on glucose and acetate, can be predicted from the models $\mathcal{M}_{glyco}$ and $\mathcal{M}_{neo}$, by means of the methods in de Jong and Page (2008). Both $\mathcal{M}_{glyco}$ and $\mathcal{M}_{neo}$ have a single stable steady state. Given that we focus on qualitative properties of the system, we are particularly interested in the difference in steady-state concentrations during glycolysis and gluconeogenesis. That is, which genes are higher or lower expressed during growth on acetate as compared to growth on glucose? Table 1 summarizes the results of the comparison of the glycolytic and gluconeogenic steady states. The predicted changes in expression correspond well to the observations (Oh et al., 2002; Liu et al., 2005; Peng and Shimizu, 2003). The observed inversion of *pykF* and *ppsA* expression is correctly reproduced, as well as the down-regulation of *gapA* during growth on acetate. The exception is the observed lower expression level of Crp in acetate-grown bacteria. This result is somewhat surprising as other measurements obtained under glucose depletion show the contrary (Ishizuka et al., 1993).

Is the correspondence with experimental data preserved when the indirect interactions are omitted from the model? In order to answer this question, we computed the steady states for $\mathcal{M}^0$, the model accounting for purely transcriptional interactions. Table 1 shows that $\mathcal{M}^0$ fails to reproduce most of the observed changes in gene expression. Indeed, according to $\mathcal{M}^0$, enzyme concentrations are independent of the specific growth condition. This confirms the importance of metabolic coupling for the adjustment of gene expression level when cells are alternatively grown on glucose and acetate.

Are the indirect interactions equally important for the dynamic response of the system? When glucose is exhausted the bacteria are able to continue growth on an alternative carbon source like acetate. Such a diauxic shift entails important changes in metabolism, as well as a reorganization of gene expression. The predicted transcriptional response of the bacteria to glucose depletion is shown in Table 2. We compare the synthesis rate of the proteins in the glycolysis steady state and in the successor state following the rapid drop in glucose levels. The synthesis rates of most proteins are seen to react immediately to the change in nutrient availability, in agreement with the experimental data of Kao et al. (2005).

The importance of metabolic coupling can be assessed by comparing the above predictions of the response of the system to the exhaustion of glucose with the predictions obtained by means of the $\mathcal{M}^0$ model. In the latter case network response is impaired, for the simple reason that in the absence of metabolic coupling the transcription factors Crp and FruR cannot sense the change in concentration of their metabolic effectors, cAMP and FBP, respectively.

In order to better understand the role of the different coupling species, we analyzed the behavior of two additional models in which one coupling species (either Crp·cAMP or free FruR) is removed at a time. The results show that the control of the gene expression response is shared between different mechanisms. Metabolic coupling through free FruR deals with the control of metabolic fluxes, via the expression of glycolytic enzymes. For instance, the key inversion in *ppsA* and *pykF* expression during a glucose-acetate diauxie is absent when free FruR is eliminated. By contrast, the control exerted via the Crp·cAMP complex proves more global, equally affecting enzymes, transcription factors, and other regulators, in agreement with the predominant role of Crp·cAMP in the control of carbon metabolism (Nanchen et al., 2008). More details on the analysis of the role of the different coupling species can be found in (Baldazzi et al., 2011).

4. CONCLUSIONS

We have used simple, qualitative models to explore the importance of metabolic coupling for the qualitative dynamics of gene regulatory networks. Our analysis of the gene regulatory network controlling carbon assimilation in *E. coli* shows that indirect interactions completely modify the predicted expression patterns following a glucose-acetate shift, transforming genes from passive, constitutively expressed elements into active players of the adaptive response of the cell. The profiles obtained when including metabolic coupling show good correspondence with the available experimental data, contrary to what is observed when the model is restricted to transcriptional regulation only. In particular, key signatures of growth on a glycolytic substrate (glucose) as compared to growth on a neoglucogenic substrate (acetate) are reproduced, such as the opposite expression levels of the enzymes PpsA and PykF.

The indirect interactions arising from metabolic coupling are found to be crucial, because they convey rapid changes in metabolism (fluxes, metabolite concentrations) that control the activity of transcriptional regulators. This allows environmental perturbations, such as the depletion of glucose, to propagate through the network and affect the expression of a large number of genes. While these results have been obtained for one particular bacterial model system, the ubiquity of metabolic coupling in regulatory networks makes our conclusions relevant for other systems as well.

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	crp	*fis*	*rpoS*	*fruR*	*gapA*	*ppsA*	*pykF*	Reference/Model
Experimental data	?	-	+	?	-	+	-	Oh et al. (2002)
	-	-	+	+		+	-	Liu et al. (2005)
						+	-	Peng and Shimizu (2003)
Model predictions	0	0	0	0	0	0	0	$\mathcal{M}^0$
	+	-	+	0	-	+	-	$\mathcal{M}_{neo}$ vs $\mathcal{M}_{glyco}$

Table 1. Predicted and observed differences in gene expression during growth on acetate vs growth on glucose. The model predictions are the differences in steady-state concentrations (+: higher, −: lower, 0: equal). The measurements concern the differences in mRNA concentrations (Oh et al., 2002; Liu et al., 2005) and protein concentrations (Peng and Shimizu, 2003). In some cases the precision of the measurements does not allow to distinguish between qualitative differences, indicated by ?.

	crp	*fis*	*rpoS*	*fruR*	*gapA*	*ppsA*	*pykF*	Reference/Model
Experimental data	-	-	-	0	-	+	0	Kao et al. (2005)
Model predictions	0	0	0	0	0	0	0	$\mathcal{M}^0$
	+	-	-	0	-	+	0	$\mathcal{M}_{glyco}$

Table 2. Predicted and observed initial responses on the transcriptional level when glucose is exhausted. The model predictions are the immediate change in the protein synthesis rate following the exhaustion of glucose, in comparison with the steady-state protein synthesis rate during glycolysis (+: higher, −: lower, 0: equal). The measurements concern the initial change in mRNA concentrations when the bacteria are transferred from a glucose to acetate medium (Kao et al., 2005).

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