Qualitative simulation of the carbon starvation response in *Escherichia coli*

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Received 23 May 2005; received in revised form 28 September 2005; accepted 4 October 2005

Abstract

In case of nutritional stress, like carbon starvation, *Escherichia coli* cells abandon their exponential-growth state to enter a more resistant, non-growth state called stationary phase. This growth-phase transition is controlled by a genetic regulatory network integrating various environmental signals. Although *E. coli* is a paradigm of the bacterial world, it is little understood how its response to carbon starvation conditions emerges from the interactions between the different components of the regulatory network. Using a qualitative method that is able to overcome the current lack of quantitative data on kinetic parameters and molecular concentrations, we model the carbon starvation response network and simulate the response of *E. coli* cells to carbon deprivation. This allows us to identify essential features of the transition between exponential and stationary phase and to make new predictions on the qualitative system behavior following a carbon upshift.

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Keywords: Qualitative modeling and simulation; Piecewise-linear differential equations; Genetic regulatory networks; Carbon starvation; Nutritional stress response; *Escherichia coli*

1. Introduction

In their natural environment, bacteria rarely encounter conditions allowing continuous, balanced growth. While environmental conditions are favorable, bacterial populations grow quickly, leading to an exponential increase of their biomass, a state called *exponential phase*. However, upon a variety of stress conditions like the depletion of a carbon source, the bacteria are no longer able to maintain fast growth rates, and the population consequently enters a non-growth state, called *stationary phase* (Fig. 1(a)). During the transition from exponential to stationary phase, each individual bacterium develops an ability to survive prolonged periods of starvation and becomes resistant to multiple stresses. For example, some Gram-positive bacteria, like *Bacillus subtilis*, can differentiate into spores (Sonenshein, 2000), whereas *Escherichia coli* and related enteric bacteria undergo other important modifications of their morphology and physiology (Fig. 1(b)) (Huisman et al., 1996). The response of bacterial cells to various stresses can be reversed and growth resumed as
soon as the environmental conditions become favorable again.

On the molecular level, the transition from exponential to stationary phase in *E. coli* involves a variety of events (Hengge-Aronis, 1996). For example, the cellular metabolism, previously aimed at maximal growth, is reoriented towards a metabolism of maintenance, and a large number of genes are induced, whose function it is to provide maximal protection against a variety of stresses (Chang et al., 2002; Chuang et al., 1993; Hengge-Aronis, 1996, Huisman et al., 1996; Ishihama, 1997; Kolter et al., 1993; Selinger et al., 2000; Tani et al., 2002). DNA topology is also affected by this process, in that the DNA, usually supercoiled during exponential phase, becomes relaxed when cells enter stationary phase (Balke and Gralla, 1987).

The morphological and physiological changes during the growth-phase transition are controlled by a complex genetic regulatory network integrating various environmental signals (Hengge-Aronis, 2000; Martinez-Antonio and Collado-Vides, 2003; Wick and Egli, 2004). Among the numerous genes, proteins, and metabolites making up this network, a class of pleiotropic transcription factors, called global regulators, plays a key role (Gottesman, 1984). Global regulators mediate the activation or repression of a large number of genes in response to changes in environmental conditions, such as nutrient deprivation. Hence, they are able to regulate operons belonging to different metabolic pathways, as well as genes involved in different cellular processes. Many transcription factors have been identified in *E. coli*, but only some of them classify as major global regulators such as CRP, RpoS, ppGpp, IHF, FNR, Foc, Lep, HNS, and ArcA (Madan Babu and Teichmann, 2003; Hengge-Aronis, 1996, 2000; Martinez-Antonio and Collado-Vides, 2003; Shen-Ort et al., 2002). These proteins are able to directly modulate the expression of up to 51% of *E. coli* genes.

DNA topology is also considered as a global regulator, since the expression of many genes has been shown to be sensitive to the DNA supercoiling level (Hatfield and Benham, 2002; Peter et al., 2004).

The ways in which these global regulators exercise their control are multifarious: they are able to regulate gene expression alone or jointly with other global or specific regulators, switching on or off target genes in a combinatorial fashion (Martinez-Antonio and Collado-Vides, 2003). They also control the expression of their own genes and the genes encoding their co-regulators through complex feedback loops. In addition, the activity of global regulators may be controlled by metabolites and signalling molecules produced in response to environmental perturbations, such as cAMP and ppGpp.

The genetic regulatory network controlling the adaptation of *E. coli* cells that occur during the transition from exponential to stationary phase has been the subject of extensive studies for decades. For example, the mechanism of action of each global regulator is well known, as well as the identity of most of the other transcription factors and target genes involved in the process (Barnard et al., 2004; Chang et al., 2002; Gottesman, 1984; Hengge-Aronis, 1996; Martinez-Antonio and Collado-Vides, 2003; Postma et al., 1996; Saiter and Ramseier, 1996). However, most studies have focused on only one or a few components of the network and currently no global view exists of the precise functioning of the network as a whole. Computer modeling and simulation tools may help to clarify how the global behavior of this kind of complex system emerges from the local interactions between its components (McAdams and Arkin, 1998). Indeed, they provide a framework for integrating available biological data at the molecular level and for predicting the behavior of the system under various environmental and physiological conditions. This not only contributes to a better understanding of the role of
the different components of the network and their interactions, but also allows the formulation of hypotheses about missing components and interactions, which may guide further experimentation.

A wide variety of mathematical formalisms for describing genetic network dynamics exists, giving rise to qualitative or quantitative, discrete or continuous, stochastic or deterministic models (de Jong, 2002). The most widely-used approach is based on ordinary differential equations (ODEs) and has a solid foundation in the kinetic theory of biochemical reactions. However, the use of chemical kinetics requires precise knowledge of biochemical reaction mechanisms, as well as quantitative information on molecular concentrations and kinetic parameters. Even in the case of well-studied systems, like the E. coli stress response network, this level of knowledge is almost never obtained (de Jong and Ropers, 2005; Gagneur and Casari, 2005). A way to deal with this problem is to use a special class of piecewise-linear (PL) differential equation models, originally proposed by Glass and Kauffman (1973).1 The state variables in the PL models correspond to the concentrations of the proteins encoded by genes in the network, while the differential equations describe the influence of each regulatory protein on the synthesis and degradation of other network components. The models use a simplified description of the regulatory interactions in terms of step functions, motivated by the non-linear, switch-like character of the interactions involved in gene expression. In addition, the use of PL differential equations facilitates the qualitative analysis of the network dynamics, thus circumventing the problem that quantitative information is usually lacking (de Jong et al., 2004b; Glass and Kauffman, 1973).

Our long-term aim is to analyze the whole network of global regulators, in order to better understand how the growth-phase transition of E. coli emerges from the interactions between these regulators. As a first step, we focus in this paper on one particular part of the network controlling the response of the cell to a well-studied stress, namely carbon starvation. Because quantitative data are usually absent, we have used a qualitative modeling and simulation method based on the PL models of genetic regulatory networks (de Jong et al., 2004b). This approach has been successfully applied to the analysis of the regulatory networks controlling the initiation of sporulation in B. subtilis (de Jong et al., 2004a) and quorum sensing in Pseudomonas aeruginosa (Usseglio Viretta and Fussenegger, 2004). Our model consists of a system of six PL differential equations, representing the evolution of the concentration of key global regulators in the carbon starvation response network. The differential equations are supplemented with 48 inequality constraints on the parameters. Simulations of the adaptation of E. coli growth in response to carbon deprivation have been performed by means of the publicly-available software tool Genetic Network Analyzer (GNA) (de Jong et al., 2003).

The predictions obtained in this way display two interesting features. First, the reorganization of the gene expression pattern during the transition from the exponential to the stationary growth phase relies on a switch in the expression of the genes fis and crp, coding for two important global regulators. Second, damped oscillations of protein concentrations occur after a carbon upshift, due to the homeostatic control of DNA topology. From a more general point of view, our mathematical model connects several functional modules in the carbon starvation response network - modules that have been usually studied in isolation - and shows how the global adaptive behavior of the cells to their environment emerges from local interactions at the molecular level inside and between modules.

In Section 2, we review our current knowledge about the network of interactions controlling the carbon starvation response in E. coli: Section 3 summarizes the qualitative modeling and simulation approach that has been used to analyze the carbon starvation response network. In Sections 3 and 4 we present a qualitative PL model of the network of key global regulators and the functional modules in which they are involved. The results of qualitative simulation are presented in Section 5, followed by a discussion of the biological implications of these results in the concluding section of the paper.

2. The carbon starvation response network

In order to model the carbon starvation response in E. coli, we have chosen to advance by a modular approach, based on evidence that the behavior of genetic regulatory networks arises from the interactions between subnetworks with a specific function (Hartwell et al., 1999; Tyson et al., 2003). The four modules that we consider are defined by three global regulators (CRP, Fis, and DNA topology), one input (a signal of carbon deprivation), and one output (the stable RNA concentration). In order to fill in the molecular details, we have used data from the extensive literature on the carbon starvation response in E. coli and computerized resources such as EcoCyc (Karp et al., 2002; Keseler et al., 2005) and

1 See Kauffman (1993), Thomas and d’Ari (1990), Thomas et al. (1995) for alternative approaches based on logical models.
Fig. 2. Network of key genes, proteins, and regulatory interactions involved in the carbon starvation response network in *Escherichia coli*. The contents of the boxes ‘CRP activation’ and ‘supercoiling’ are detailed in Fig. 3. The graphical conventions (Kohn, 2001) are explained in the legend.

RegulonDB (Salgado et al., 2004). Fig. 2 depicts the components of this network and their interactions, using conventions proposed by Kohn (2001).

The aim of this paper is to give a minimal representation of the adaptation of the growth of the cell to a carbon stress. It includes neither RpoS nor ppGpp, both important regulators of stationary phase. As a consequence, certain other aspects of the carbon stress response cannot be accounted for by the model, as will be explained below. Future versions of the model will achieve an increasingly-broader coverage of the network of interactions involved in the carbon stress response.

The first network module concerns the input of the system. In adaptation processes of the kind considered here, the relationship between sensing external stimuli and expressing appropriate genes involves first of all transporters and signal-transduction mechanisms, driving protein activation and metabolite production. In *E. coli*, the signal indicating carbon source depletion is transmitted by the activation of the adenylate cyclase enzyme (Cya) and the subsequent production of cAMP, a small metabolite that binds the dimeric form of the protein CRP (cAMP receptor protein) (Saier and Ramseier, 1996). The thus activated enzyme is able to efficiently produce cAMP from ATP (Fig. 3(a)). The above signal-transduction process involves the phosphotransferase system (PTS), responsible for the regulation of the transport of sugars (Postma et al., 1996). The PTS senses the lack of a carbon source and transmits the information to other cellular processes through a cascade of phosphorylation reactions triggering the activation of adenylate cyclase. Because in this paper we focus on the downstream effect of the signal, the PTS signal-transduction pathway will not be modeled in detail (some examples of models of this system can be found in the literature, e.g., Francke et al. (2003), Kremling et al. (2001), Rohwer et al. (2000), Thattai and Shraiman (2003)). Instead, we simplify the signal-transduction pathway by considering a signal indicating a lack of carbon source (abbreviated to signal) that directly activates Cya under carbon starvation conditions (Fig. 2).

The second network module comprises three genes: crp, cya, and fis. The global regulator CRP is the target of the signal-transduction pathway: as soon as the cAMP metabolite is produced, it binds CRP, thus activating the transcription factor and allowing it to regulate gene expression (Harman, 2001; Lawson et al., 2004). The cAMP-CRP complex controls the expression of two kinds of genes. The first category is composed of genes directly involved in the adaptive response to the deprivation of a carbon source: for example, it activates the synthesis of catabolic enzymes, thus amplifying the potential of the cells to make use of alternative sources of carbon (Gosset et al., 2004; Saier and Ramseier, 1996). cAMP-CRP also controls the expression of genes involved in changes of cellular morphology and motility. The second category of cAMP-CRP-responsive genes are those encoding global regulators like the proteins Fis and CRP itself (Gosset et al., 2004; Martinez-Antonio and Collado-Vides, 2003). In addition to its autoregulation, the crp gene is regulated by Fis (Gonzalez-Gil et al., 1998). The latter protein is responsible for the control of a large number of genes involved in the cellular metabolism (Gonzalez-Gil et al., 1996), and is specifi-
Fig. 3. (a) Control of the production of the cAMP·CRP complex. A carbon starvation signal (Signal) activates the adenylate cyclase (Cya), thus allowing the production of cAMP from ATP. The small molecule binds the global regulator CRP and enables it to regulate gene expression. (b) Control of the DNA topology. The level of DNA supercoiling is mainly the result of a balance between the activity of GyrAB, which supercoils the DNA structure, and TopA, which relaxes the DNA.

cally produced at a very high level during exponential phase (Ali Azam et al., 1999; Ball et al., 1992). Fis and cAMP·CRP mutually repress each other’s expression.

The third network module contains genes encoding the proteins GyrAB, TopA, and Fis involved in the control of DNA topology. Bacterial chromosomes in living cells are generally in a negatively supercoiled rather than relaxed form (Drlica, 1990; Pettijohn, 1996). Negative supercoiling is an important modulator of all processes involving DNA as a substrate, that is, processes melting, bending, or distorting the molecule. In particular, it influences the transcription of many genes, either by realigning the anchor sites of the RNA polymerase in the promoter region, thus favoring the recognition of the latter by the enzyme, or by facilitating local DNA unwinding, which is required for transcription initiation (Hatfield and Benham, 2002). The control of DNA supercoiling mainly involves two bacterial topoisomerases into the DNA molecule in an ATP-dependent manner, the product of the gene topA, the topoisomerase TopA, removes negative supercoils, without requiring ATP. The supercoiling level is tightly autoregulated in bacteria: an increase of negative supercoiling by GyrAB is compensated by the activation of topA expression, which leads to increased levels of TopA, and thus relaxation of excessive negative supercoiling (Hatfield and Benham, 2002). Conversely, a decrease in negative supercoiling releases inhibition of gyrA and gyrB promoters and thus increases GyrAB production, thereby restoring the physiological level of DNA supercoiling. The protein Fis also takes part in this fine-tuning process of homeostatic control (Menzel and Gellert, 1983), since an increase of negative supercoiling stimulates Fis expression, which represses GyrAB production on the one hand, and stimulates TopA expression on the other hand (Schneider et al., 1999, 2000; Travers et al., 2001). Since the genes gyrA and gyrB encoding GyrAB are regulated in a similar way (Schneider et al., 1999), we will here simplify the discussion by considering the GyrAB complex as the product of a single gene, gyrAB. Since GyrAB requires ATP for its activity, it is likely that cellular energy also affects DNA topology and therefore controls expression of supercoiling-sensitive genes. However, in this report, we will assume DNA supercoiling to be only dependent on the amounts of GyrAB and TopA.

The last network module controls cellular growth and thus represents the output of the system. It is composed of genes encoding Fis and stable RNAs. The latter are considered as representative of the cell’s growth state, since cells growing and dividing need huge amounts of stable RNAs in order to translate mRNAs into proteins (Bremer and Dennis, 1996; Keener and Nomura, 1996). Conversely, cells in growth arrest have a limited translational activity and consequently no longer require high levels of stable RNAs. In the E. coli genome, the genes coding for these RNAs are organized in seven rrn operons with the same overall structure, each being composed of the three genes for ribosomal RNA and at least one for transfer RNA (Keener and Nomura, 1996; Paul et al., 2004; Schneider et al., 2003). The expression of these RNAs is strongly stimulated by the Fis protein (Paul et al., 2004; Schneider et al., 2003). As production of stable RNAs is the hallmark of cellular growth, we will consider the cells to be in stationary phase when the rrn concentration is low.

How does the switch between growing or slowing down growth emerge from the interconnections between the four network modules? The functioning of the individual modules can be understood intuitively to a certain extent. However, it is not known how the
global behavior of the system emerges from the local interactions inside and between modules. A closer look at Fig. 2 reveals that it contains a number of positive and negative feedback loops that are suspected to play a fundamental role in controlling the growth transition. Examples of these feedback loops are the homeostatic control of DNA supercoiling and the cross-inhibition of fis and crp. The interactions making up these feedback loops are critical for the network connectivity, since they link the different modules together. In order to better understand the role of the feedback loops for the system dynamics, we have built a mathematical model of the regulatory network and simulated the behavior of the system.

To date no comprehensive mathematical models of the carbon starvation response network in Fig. 2 have been proposed, although some particularly well-studied parts of the network have been the subject of modeling studies. We mention, for example, the description of the DNA supercoiling level as a function of the cellular energy charge (Jensen et al., 1999; Snoep et al., 2002), the regulation of the transcription efficiency by CRP (Kremling and Gilles, 2001), as well as the carbohydrate uptake by the phosphotransferase system (Francke et al., 2003; Kremling et al., 2001; Rohwer et al., 2000; Thattai and Shraiman, 2003). However, the dynamics of the entire nutritional stress response network has never been studied, the principal reason being that, except for the above-mentioned network parts, quantitative data on kinetic parameters and molecular concentrations are not usually available. In order to deal with this lack of data, we use in this report a qualitative simulation method, described in Section 3. This approach has allowed us to gain an understanding of how E. coli cells adapt their growth to different environmental conditions.

3. Qualitative simulation of genetic regulatory networks

The qualitative simulation method described in (de Jong et al., 2004b) is based on a class of piecewise-linear (PL) differential equation models that provide a coarse-grained description of genetic regulatory networks. The PL models have mathematical properties that allow qualitative predictions on the steady-state and transient behavior of the system to be made. Below, we give a brief summary of the method, focusing on the description of genetic regulatory networks by PL models and the predictions obtained from the models through qualitative simulation.

3.1. Piecewise-linear models of genetic regulatory networks

The dynamics of genetic regulatory networks can be modeled by a class of piecewise-linear (PL) differential equations originally proposed by Glass and Kauffman (1973), and generalized by Mestel et al. (1995). The equations have the form

\[ x_i = f_i(x) - g_i(x)x_i, \quad x_i \geq 0, \ 1 \leq i \leq n, \]

(1)

where \( x = (x_1, \ldots, x_n) \) is a vector of cellular protein concentrations. The state equations (1) define the rate of change of the concentration \( x_i \) as the difference of the rate of synthesis \( f_i(x) \) and the rate of degradation \( g_i(x) \) of the protein.

The function \( f_i : \mathbb{R}^n_+ \rightarrow \mathbb{R}_+ \) expresses how the rate of synthesis of the protein encoded by gene \( i \) depends on the concentrations \( x \) of proteins in the cell. It is defined as

\[ f_i(x) = \sum_{l \in 0} k_{il} b_{il}(x), \]

(2)

where \( k_{il} \) is a rate parameter (\( k_{il} > 0 \)), \( b_{il} : \mathbb{R}^n_+ \rightarrow [0, 1] \) a regulation function, and \( L \) a possibly empty set of indices of regulation functions. The function \( g_i \) describes the regulation of protein degradation, as well as its disappearance through growth dilution. It is defined analogously to \( f_i \) except that we demand that \( g_i(x) \) be strictly positive. In addition, in order to formally distinguish degradation rates from synthesis rates, we will denote the former by \( \gamma \) instead of \( \kappa \). Notice that with the above definitions of \( f_i \) and \( g_i \), the state equations (1) are piecewise-linear (PL).

A regulation function \( b_{il} \) describes the logic of gene regulation (Plahte et al., 1998; Thomas and d’Ari, 1990). More precisely, it describes the conditions under which the protein encoded by gene \( i \) is synthesized (degraded) at a rate \( \kappa_i (x_{i_1}, x_{i_2}) \). These conditions are formulated as expressions of step functions \( s^+ : x^+ : \mathbb{R}^2 \rightarrow [0, 1] \)

\[ s^+(x_j, \theta) = \begin{cases} 1, & x_j > \theta_j, \\ 0, & x_j < \theta_j, \end{cases} \]

and \( s^-(x_j, \theta) = 1 - s^+(x_j, \theta) \),

(3)

where \( x_j \) is an element of the state vector \( x \), and \( \theta_j \) a constant denoting a threshold concentration (\( \theta_j > 0 \)). Notice that step functions \( s^+(x_j, \theta) \) and \( s^-(x_j, \theta) \) are not defined for \( x_j = \theta_j \), so neither are the regulation functions in which they occur (but see Section 3.2). We use regulation functions that are the arithmetic equivalent of logical functions, as described in (Plahte et al., 1998).
The PL models can be extended to take into account input variables \( u = (u_1, \ldots, u_n) \), representing the concentration of proteins and small molecules whose synthesis and degradation are regulated outside the system. This leads to models of the form:

\[
\dot{x}_i = f_i(x, u) - g_i(x, u) \xi_i,
\]

\( x_i \geq 0, u_j \geq 0, 1 \leq i \leq n, 1 \leq j \leq m, \) \( (4) \)

In what follows, we will assume that the input variables are constant, i.e., \( u = 0 \). As a consequence, (4) can be reduced to (1) without loss of generality, by prior evaluation of the step-function expressions in which input variables occur.

3.2. Mathematical analysis of piecewise-linear models

The dynamical properties of the PL models can be analyzed in the \( n \)-dimensional phase space box \( \Omega = \Omega_1 \times \ldots \times \Omega_n \), where every \( \Omega_i \), \( 1 \leq i \leq n \), is defined as

\[
\Omega_i = \{ x_i \in \mathbb{R} \mid 0 \leq x_i \leq \text{max}_i \},
\]

and \( \text{max}_i \) is a parameter denoting a maximum concentration for the protein.

Given that the protein encoded by gene \( i \) has \( p_i \) threshold concentrations, the \( (n-1) \)-dimensional threshold hyperplanes \( x_i = \theta_i^{D} \), \( 1 \leq p_i \leq p_n \), partition \( \Omega \) into hyperrectangular regions that are called domains. More precisely, a domain \( D \subseteq \Omega \) is defined by \( D = D_1 \times \ldots \times D_n \), where every \( D_i \), \( 1 \leq i \leq n \), is given by one of the equations below:

\[
D_1 = \{ x_1 \mid 0 \leq x_1 < \theta_1^{D} \},
\]

\[
D_2 = \{ x_2 \mid x_1 = \theta_1^{D} \},
\]

\[
D_3 = \{ x_3 \mid x_1 < x_2 < \theta_2^{D} \},
\]

\[
\ldots
\]

\[
D_n = \{ x_n \mid \theta_n^{D} \leq x_n \leq \text{max}_n \}. \]

If for a domain \( D \), there are some \( i, j \), \( 1 \leq i \leq n, 1 \leq j \leq p_i \), such that \( D_i = \{ x_i \mid x_i = \theta_i^{D} \} \), then \( D \) is called a switching domain. Otherwise, \( D \) is called a regulatory domain.

When evaluating the step-function expressions in (2) in a regulatory domain, \( f_i(x) \) and \( g_i(x) \) reduce to sums of rate constants. More precisely, in every regulatory domain \( D \subseteq \Omega \), \( f_i(x) \) reduces to some \( \mu_i^{D} \in M_i \equiv \{ f_i(x) \mid 0 \leq x \leq \text{max}_i \} \), and \( g_i(x) \) to some \( p_i^{D} \in N_i \equiv \{ g_i(x) \mid 0 \leq x \leq \text{max}_i \} \). \( M_i \) and \( N_i \) collect the synthesis and degradation rates of the protein in different domains of \( \Omega \). It can be easily shown that all trajectories in \( D \) monotonically converge towards a so-called target equilibrium, lying at the intersection of the \( (n-1) \)-dimensional threshold hyperplanes \( x_i = \mu_i^{D}/p_i^{D} \), \( 1 \leq i \leq n \) (Glass and Kauffman, 1973; Mestl et al., 1995; Snoussi, 1989). The target equilibrium level \( \mu_i^{D}/p_i^{D} \) of the protein concentration \( x_i \) gives an indication of the strength of gene expression in \( D \). Call \( \Phi(D) = \{(\mu_1^{D}/p_1^{D}, \ldots, \mu_n^{D}/p_n^{D})\} \) the target equilibrium set of \( D \).

If \( \Phi(D) \cap D \neq \emptyset \), then all solutions in \( D \) asymptotically approach the target equilibrium, which is then a stable equilibrium point of the system. If \( \Phi(D) \cap D = \emptyset \), the solutions will leave \( D \) at some point.

In switching domains, \( f_i(x) \) and \( g_i(x) \) are not defined in general, because some concentration variables assume a threshold value. Moreover, due to the use of step functions, \( f_i(x) \) and \( g_i(x) \) may be discontinuous at the \( (n-k) \)-dimensional threshold hyperplane in which the domain is contained \( 1 < k < n \). In order to cope with this problem, the system of differential equations (1) is extended into a system of differential inclusions, following an approach inspired by Filippov (de Jong et al., 2004b; Filippov, 1988; Gouzé and Sari, 2003).

Using this generalization, it can be shown that, in the case of a switching domain \( D \), the solution trajectories either cross \( D \) instantaneously or converge towards a target equilibrium set \( \Phi(D) \) located in the threshold hyperplane containing \( D \). Here, \( \Phi(D) \) is the smallest hyperrectangle containing the target equilibria of the regulatory domains that have \( D \) in their boundary, intersected with the threshold hyperplane containing \( D \). In the case of switching domains, \( \Phi(D) \) is generally not a single point. If \( \Phi(D) \cap D = \emptyset \), all solutions will leave \( D \) at some point. On the other hand, if \( \Phi(D) \cap D \neq \emptyset \), there exist solutions in \( D \) that reach or asymptotically approach the target equilibrium set \( \Phi(D) \) as \( t \to \infty \).

Every \( \Phi \in \Phi(D) \cap D \) is an equilibrium point of the system. Whether this equilibrium point is stable or unstable must be determined through further analysis (Casey et al., in press).

3.3. Qualitative description of dynamics of piecewise-linear models

The mathematical framework presented in Section 3.2 suggests an intuitive qualitative description of the dynamics of regulatory systems described by PL models (1). This description is based on a discrete abstraction of the state of the system, a so-called qualitative state, consisting of the domain \( D \) in which the system resides and the position of the target equilibrium set \( \Phi(D) \) with respect to \( D \). A qualitative state thus captures the local dynamics of the system. There
exists a transition between two qualitative states \( \text{QS} \) and \( \text{QS}' \), corresponding to contiguous domains \( D \) and \( D' \), if some solution trajectories starting in \( D \) reach \( D' \), without passing through an intermediate domain. The sets of qualitative states and transitions between qualitative states define a state transition graph, summarizing the qualitative dynamics of the regulatory system.

The state transition graph may contain one or more qualitative equilibrium states, each of which corresponds to an equilibrium point of the system. A path in the state transition graph is called a qualitative behavior. It describes how the bounds on protein concentrations evolve over time, according to the sequence of transitions between qualitative states. A cyclic qualitative behavior, called a qualitative cycle, may correspond to a limit cycle or to trajectories spiraling towards or away from an equilibrium point. The set of qualitative states from which a qualitative equilibrium state or qualitative cycle is reachable forms its attraction set.

The qualitative nature of the state transition graph is well-adapted to measurement techniques in genomics, which currently have limited quantitative precision, but are able to detect qualitative changes in gene expression over time.

3.4 Qualitative piecewise-linear models

Most of the time, precise numerical values for the threshold and rate parameters in a PL model are not available. However, instead of specifying precise numerical values, it is often possible to supplement the state equations with inequality constraints on the parameter values. The inequality constraints express weak, but reliable information on the regulatory interactions that can be inferred from biological data. The resulting, so-called qualitative PL model subsumes a set of quantitative PL models.

The first type of constraint, the threshold inequalities, are obtained by ordering the \( \mu_i \) threshold concentrations of the protein encoded by gene \( i \), i.e.,

\[
0 < \frac{\mu_i}{v_i} < \frac{\mu_i}{v_i} < \frac{\mu_i}{v_i} < \cdots < \frac{\mu_i}{v_i} < \max,
\]

The threshold inequalities determine the partitioning of \( S \) into regulatory and switching domains.

The second type of constraint, the equilibrium inequalities, order the possible target equilibrium levels of \( S \), in different regulatory domains \( D \) with respect to the threshold concentrations. Biologically speaking, the equilibrium inequalities define the strength of gene expression in the domain in a qualitative way, on the scale of ordered threshold concentrations. More precisely, for every \( \mu_i \in M_i, v_i \in N_i \), and \( \mu_i, v_i \neq 0 \), we specify one of the following pairs of inequalities:

\[
0 < \frac{\mu_i}{v_i} < \theta_1^i,
\]

\[
\theta_1^i < \frac{\mu_i}{v_i} < \theta_2^i,
\]

\[
\vdots
\]

\[
\theta_n^i < \frac{\mu_i}{v_i} < \max.
\]

The equilibrium inequalities constrain the relative position of \( D \) and its target equilibrium set \( \Psi(D) \).

The models of genetic regulatory networks treated by the simulation method consist of state equations (1), supplemented by parameter inequalities (7) and (8). Every such qualitative PL model corresponds to a set of quantitative PL models consisting of state equations (1) and a particular combination of numerical parameter values consistent with the parameter inequalities. It has been shown that in the region of the parameter space defined by the inequalities in the qualitative PL model, all quantitative PL models yield the same state transition graph (de Jong et al., 2004b). This graph can be efficiently computed from the inequality constraints by symbolic instead of numerical means.

3.5 Qualitative simulation

A state transition graph may become exceedingly large, as the number of domains, and hence qualitative states, grows exponentially with the dimension of the system. For many purposes, it is sufficient to know which qualitative states are reachable from a given initial qualitative state, that is, which qualitative behaviors the system can exhibit when initially being in this state. The algorithm for qualitative simulation described in (de Jong et al., 2004b) generates the reachable part of a state transition graph from a qualitative PL model and an initial domain.

The state transition graph generated through qualitative simulation is a prediction of the qualitative dynamics of the system. We have demonstrated that this state transition graph covers all qualitative behaviors permitted by any quantitative PL model subsumed by the qualitative PL model (de Jong et al., 2004b). That is, whatever the exact numerical values for the parameters may be, if these values are consistent with the threshold and equilibrium inequalities specified in the qualitative PL model, the qualitative properties of the solution are described by a sequence of states in the state transition graph. The converse is not true: the state transition graph resulting from a qualitative simulation may contain qualitative
Fig. 4. Screenshot of the graphical user interface VisualGNA of the qualitative simulator GNA: (a) model variables and initial conditions for the *E. coli* carbon starvation network, (b) influence graph describing the direct or indirect interactions between the network components, (c) specification of the PL differential equation and parameter inequalities for a variable, (d) fragment of the state transition graph produced by simulation from the initial conditions, and (e) qualitative temporal evolution of some model variables.

behaviors that are permitted by no quantitative PL model subsumed by the qualitative PL model.

The simulation method has been implemented in Java 1.4, in a program called GNA (Genetic Network Analyzer) (de Jong et al., 2003). In order to facilitate its use, the program is equipped with a graphical user interface, called VisualGNA. The interface includes a model editor, which integrates the specification of models of genetic regulatory networks with the simulation of the behavior of the network and the biological interpretation of the simulation results. The model editor structures and facilitates the specification of the differential equations and inequality constraints by means of check boxes, item lists, formatted text fields, and completion keys. In addition, it performs syntactic and semantic checks to guarantee the consistency and completeness of information entered by the user. VisualGNA also supports the visual analysis of state transition graphs by allowing the user, among other things, to zoom in on state transition graphs, to reduce or expand state transition graphs, and to highlight qualitative states in the graph satisfying certain user-specified criteria. In order to analyze large and complex state transition graphs in detail, GNA allows the simulation results to be exported to model-checking tools (Batt et al., 2005). Fig. 4 contains a screenshot of GNA, showing the results of a qualitative simulation of the *E. coli* carbon starvation response network (Section 6).

4. Modeling of interactions in the carbon starvation response network

In this section, we describe how the different types of interactions that play a role in the carbon starvation response can be modeled by means of PL differential equations using step functions. More particularly, we...
cover the cases where gene expression is controlled by a transcriptional regulator (Section 4.1), by a transcriptional regulator modified through a signal-transduction pathway (Section 4.2), and by DNA topology (Section 4.3). The resulting model components are combined in Section 5, where a state equation for each of the proteins or RNAs in the carbon starvation response network of Fig. 2 is given.

### 4.1. Transcriptional regulator

The control of the expression of stable RNAs by the global regulator Fis is an example of gene expression regulation by a transcriptional activator. During exponential phase, when the demand for protein synthesis is high, promoters of the seven \( rrn \) operons account for more than 60% of the cellular transcription products (Bremer and Dennis, 1996). The high activity of these promoters has been related to their stimulation by the protein Fis (Appleman et al., 1998; Nilsson et al., 1992; Zhang and Bremer, 1996; Zhang et al., 2002). In particular, the protein activates transcription by binding to regulatory sites and stabilizing the interaction between RNA polymerase and ribosomal promoters in a cooperative manner (Aiyar et al., 2002; Bokal et al., 1995; Hirvonen et al., 2001; Paul et al., 2004; Schneider et al., 2003; Zhi et al., 2003). The regulatory mechanism, shown in Fig. 5(a), can be modeled by means of a sigmoidal function, the Hill rate law (Appendix A.1, see also Dennis et al. (2004) for a detailed, kinetic model of the control of RNA synthesis). Fig. 6(a) shows the activity of the \( rrn \) promoter, and hence the rate of synthesis of stable RNAs, as a function of the cellular concentration \( x_{fis} \) of Fis. Below a certain threshold concentration of Fis, the stable RNA genes are poorly expressed, whereas above this threshold their expression reaches its maximum level.

The sigmoid curve in Fig. 6(a) can be approximated by a step function. The rate of expression of stable RNAs is then expressed in the following manner:

\[
f_{rrn}(x_{fis}) = \kappa_{rrn}s + (x_{fis}, \theta_{3fis}^{fis}).
\]

(9)

where \( \kappa_{rrn} \) and \( \theta_{3fis}^{fis} \) are constants denoting the synthesis rate of stable RNAs and the threshold concentration of Fis, respectively. The function \( f_{rrn}(x_{fis}) \) implies that stable RNAs are expressed at a rate \( \kappa_{rrn} \), if \( x_{fis} > \theta_{3fis}^{fis} \), whereas they are not expressed if \( x_{fis} < \theta_{3fis}^{fis} \).

In addition to activating \( rrn \) production, Fis has been shown to repress its own expression, by binding to the promoter region of the gene (Fig. 5(b)) (Ball et al., 1992; Ninnemann et al., 1992; Pratt et al., 1997). As in the previous example, the activity plot of \( fis \) is a sigmoidal curve (Fig. 6(b)). However, expression of this gene is now a decreasing function of the Fis concentration. Moreover, the curve is shifted to a higher concentration range, which reflects the fact that Fis binds more weakly to its own promoter region than to that of \( rrn \). The curve in Fig. 6(b) can be approximated by the following step function:

\[
f_{fis}(x_{fis}) = \kappa_{fis}s - (x_{fis}, \theta_{5fis}^{fis}).
\]

(10)
where $\kappa_{fis}$ is a synthesis rate, $\theta_{fis}$ a threshold concentration, and $\theta_{fis} > \theta_{fis}^c$.

In reality, the regulation of the expression of $rrn$ and $fis$ presented in Fig. 6 is even more complex: transcription of the gene $fis$ is also controlled by the cAMP CRP complex, while the synthesis of stable RNAs occurs from two instead of one promoter. This added complexity has been omitted for the purpose of the above examples, but will be included in Section 5.

4.2. Transcriptional regulator activated through a signal-transduction pathway

As seen in Section 2, the global regulator CRP is activated through a signal-transduction pathway, in response to the lack of a carbon source. More precisely, CRP forms a complex with cAMP as soon as this metabolite is synthesized from ATP by the active form of adenylate cyclase Cya (Harman, 2001; Saier and Rameseier, 1996). The cAMP-CRP complex is able to regulate the expression of a large number of genes. It interacts with specific sequences in cAMP responsive promoters, and either promotes transcriptional initiation by stabilizing RNA polymerase or represses initiation of transcription, mostly through competitive binding to the promoter (Busby and Kolb, 1996; Gosset et al., 2004; Lawson et al., 2004; Saier and Ramseier, 1996). The cAMP is synthesized from ATP by the active form of adenylate cyclase Cya (Harman, 2001; Saier and Ramseier, 1996). The cAMP-CRP complex is able to control its own production by activating transcription signal is present, and to 0 if it is absent. Then the plots in Fig. 8 give rise to the following step-function approximation of the synthesis rate of $crp$:

$$f_{crp}(x_{crp}, x_{cya}, u_s) = \kappa_{crp} \cdot x_{crp} \cdot (\theta_{crp}^u)^x_{crp} \times x_{cya} \cdot (\theta_{cya}^u)^x_{cya}. \quad (11)$$

where $\kappa_{crp}$ is a rate parameter, while $\theta_{crp}^u$ and $\theta_{cya}^u$ are threshold parameters for $x_{crp}$ and $x_{cya}$, respectively. The function $f_{crp}(x_{crp}, x_{cya}, u_s)$ formalizes the regulatory logic shown in the plots. In particular, if the carbon starvation signal is present ($x_{crp}(u_s, \theta) = 1$), then gene $crp$ is expressed at a rate $x_{crp}$, if and only if $x_{crp} > \theta_{crp}^u$ and $x_{cya} > \theta_{cya}^u$. If the carbon starvation signal is absent, then $crp$ is not expressed.

At a higher concentration, the cAMP CRP complex inhibits cya expression (Fig. 7(b)) (Aiba, 1985; Aiba et al., 1983; Inada et al., 1996; Kawamukai et al., 1985; Mori and Aiba, 1985). Repeating the above analysis results in the cya activity plots presented in Fig. 9. The sigmoidal curve in Fig. 9(b) is reversed due to the repressive effect of cAMP CRP on cya expression. Moreover, it is shifted to a different region of the concentration space, because a high level of the cAMP CRP complex is
required to repress the cya promoter (Saier and Ramseier, 1996). The regulatory logic of cya is now approximated by

\[ f_{cya}(x_{crp}, x_{cya}, u) = \kappa_{cya}(1 - s^{+}(x_{crp}, \theta_{3}^{crp}) \times s^{+}(x_{cya}, \theta_{3}^{cya})) \times s^{+}(u, \theta_{s})), \tag{12} \]

with \( \theta_{1}^{crp} < \theta_{3}^{crp} \) and \( \theta_{1}^{cya} < \theta_{3}^{cya} \).

The step-function expressions (11) and (12) describe only part of the regulatory logic for the genes crp and cya. A complete description of the control of expression of these genes will be given in Section 5.

4.3. DNA supercoiling

DNA supercoiling constrains the topological structure of DNA, and thereby influences gene expression through a complicated mechanism. DNA topology is controlled by two enzymes, GyrAB and TopA, but responds to other physical and chemical parameters factors as well (Drlica, 1990; Hatfield and Benham, 2002; Reece and Maxwell, 1991). Depending on the relative concentration of the two enzymes, DNA supercoiling can reach different levels in the cell: in the presence of high amounts of GyrAB, many negative supercoils are introduced into the DNA, whereas these are relaxed when the TopA concentration is high. The supercoiling level controls the expression of the fis and gyrAB genes, as shown in Fig. 10.

The mechanism of the control of gene expression by DNA supercoiling is particularly difficult to model because the action of the two bacterial topoisomerases occurs through chemical reactions that produce a physical effect, a change in the helical twist of DNA (Hatfield and Benham, 2002). Existing mathematical models of DNA supercoiling have studied one of these aspects in isolation, adopting either a biophysical or a biochemical point of view. On the one hand, they have described, for example, the local melting of torsionally-stressed DNA (Benham, 1979) or the elastic deformation of DNA.
Fig. 10. Regulation of the expression of the genes (a) fis and (b) gyrAB by the DNA supercoiling level.

Benham, 1977; Yang et al., 2000). On the other hand, metabolic control analysis has been applied to the homeostatic control of DNA supercoiling by GyrAB and TopA (Jensen et al., 1999; Snoep et al., 2002). In order to integrate the physical and biochemical points of view, we have derived an empirical model that is consistent with available data on GyrAB and TopA action and on transcription regulation by DNA topology. The model takes into account the fact that the ratio of the GyrAB and TopA concentrations determines the DNA supercoiling level. In addition, it satisfies the observation that the supercoiling level stimulates the initiation of transcription by RNA polymerase through its capacity to locally unwind DNA in the promoter region, giving rise to a sigmoidal response curve (Hatfield and Benham, 2002) (Appendix A.3). This enables us to calculate the fis promoter activity as a function of the GyrAB and TopA concentrations, as shown in the activity plot in Fig. 11(a). The plot expresses the competition between the two topoisomerases: the activity of fis increases with increasing concentrations of GyrAB and decreasing concentrations of TopA, in agreement with reported data (Schneider et al., 2000).

Similar to the approach followed for the cAMP·CRP example, the sigmoidal surface can be approximated by means of step functions, yielding the following expression for the rate of synthesis from the fis promoter:

\[ f_{\text{fis}}(x_{\text{gyrAB}}, x_{\text{topA}}) = \kappa_{\text{fis}} s^+ (x_{\text{gyrAB}}, \theta_{\text{gyrAB}}^1) \times s^-(x_{\text{topA}}, \theta_{\text{topA}}^2), \]

(13)

where \( \kappa_{\text{fis}} \) is a synthesis rate, and \( \theta_{\text{gyrAB}}^1 \) and \( \theta_{\text{topA}}^2 \) are threshold concentrations of GyrAB and TopA, respectively. The function \( f_{\text{fis}}(x_{\text{gyrAB}}, x_{\text{topA}}) \) states that Fis is expressed at a rate \( \kappa_{\text{fis}} \), if \( x_{\text{gyrAB}} > \theta_{\text{gyrAB}}^1 \) and \( x_{\text{topA}} < \theta_{\text{topA}}^2 \). If this condition is not satisfied, the gene is not expressed.

When DNA supercoiling has reached a higher level, it is able to regulate other genes, such as gyrAB (Fig.
10(b) (Menzel and Gellert, 1983; 1987b; Snoep et al., 2002). Using the above-mentioned empirical model to infer the activity plots, we obtain a reverse sigmoidal surface, due to the fact that DNA supercoiling decreases gyrAB expression (Fig. 11(b)). In addition, the surface is shifted to a different region of the concentration space, reflecting the higher level at which DNA supercoiling regulates gyrAB. The approximation of the activity plot using step functions now becomes:

\[
f_{\text{cyt}}(u_{\text{cyt}}, x_{\text{gyrAB}}, x_{\text{topA}}) = x_{\text{gyrAB}} (1 - s^2(x_{\text{gyrAB}}, \theta_{\text{gyrAB}})) \\
\quad \times s^2(x_{\text{topA}}, \theta_{\text{topA}}),
\]

with \(\theta_{\text{gyrAB}}^1 < \theta_{\text{gyrAB}} < \theta_{\text{gyrAB}}^2\) and \(\theta_{\text{topA}}^1 < \theta_{\text{topA}} < \theta_{\text{topA}}^2\).

In reality, the regulation of fis and gyrAB is more complex than suggested by the above examples (Fig. 2). A complete description of the control of these genes will be given in Section 5.

5. Modeling of the carbon starvation response network

In order to model the carbon starvation response network of Fig. 2, the partial description of the interactions involved in gene regulation in Section 4 will be combined and completed. We define the state equations and parameters for the genes involved in each of the four modules: the input module (Section 5.1), the CRP activation module (Section 5.2), the DNA topology module (Section 5.3), and the output module (Section 5.4). The equations and inequalities making up the model are summarized in Fig. 12.

5.1. Input module

A signal-transduction pathway involving the PTS system is responsible for transmitting the information that a particular carbon source is no longer available to the processes taking place inside an E. coli cell (Postma et al., 1996). In particular, it drives activation of the adenylyl cyclase (Cya) responsible for the synthesis of cAMP. As explained in Section 2, we simplify the signal-transduction pathway, for which detailed kinetic models exist (Francke et al., 2003; Kremling et al., 2004; Robker et al., 2000; Thattai and Shraiman, 2003; Wang et al., 2001) to a carbon starvation signal that directly activates Cya. It is associated with the input variable \(u_i\), which is considered to be constant over the time interval of interest, that is,

\[
u_i = u_i.
\]

We define a single threshold for Signal, \(\theta_i\), giving the following inequalities:

\[0 < \theta_i < \max x_i\]

If the step function \(s^2(u_i, \theta_i)\) evaluates to 1 (0), then Signal is said to be present (absent). The presence of Signal denotes conditions of carbon deprivation that stimulates the entry into stationary phase.

5.2. CRP activation module

The CRP activation module is composed of the proteins Cya, CRP, and Fis. When activated by Signal, Cya produces the metabolite cAMP, that binds CRP and thus enables it to regulate gene expression (Harman, 2001; Postma et al., 1996; Saier and Ramsier, 1996).

The gene \(\text{crp}\) is expressed from two promoters, P1 and P2 (Alba, 1983; Gonzalez-Gil et al., 1998). Their regulation accounts for the control of the availability of carbon compounds such as glucose (Ishizuka et al., 1994, 1993; Saier and Ramsier, 1996). The protein Fis inhibits both promoters (Gonzalez-Gil et al., 1998), whereas the complex cAMP-CRP controls only promoter P1, through a regulatory mechanism that is still unclear. The available data show that the complex is able to both repress and activate \(\text{crp}\) P1 by binding to regulatory sites around the promoter (Alba, 1983; Hanamura and Aiba, 1991, 1992; Ishizuka et al., 1994; Okamoto and Freundlich, 1986; Okamoto et al., 1988). However, in order to simplify, we omit the negative control of \(\text{crp}\) P1, because this mechanism only plays a role during the exponential growth phase, when the concentration of CRP is low (Ishizuka et al., 1994).

We denote by \(\kappa_{\text{crp}}\) the background synthesis rate from promoter P1 during exponential growth, by \(\kappa_{\text{crp}}^2\) the synthesis rate induced by derepression of this promoter, and by \(\kappa_{\text{crp}}^3\), the synthesis rate from promoter P2. With a degradation rate equal to \(\gamma_{\text{crp}}\), we obtain the following state equation for \(x_{\text{crp}}\):

\[x_{\text{crp}} = \kappa_{\text{crp}}^1 + \kappa_{\text{crp}}^2 s^2(x_{\text{fis}}, \theta_{\text{fis}}) s^2(x_{\text{crp}}, \theta_{\text{crp}}) \\
\quad \times s^2(x_{\text{topA}}, \theta_{\text{topA}}) + (\kappa_{\text{crp}}^3 - \gamma_{\text{crp}}) x_{\text{crp}}.
\]
Fig. 12. State equations and parameters inequalities for the carbon starvation response network in Fig. 2. The model has six state variables corresponding to the concentrations of key proteins and stable RNAs, as well as one exogenous variable denoting the presence of a carbon starvation signal: $x_{crp}$ (CRP), $x_{cya}$ (Cya), $x_{fis}$ (Fis), $x_{gyrAB}$ (GyrAB), $x_{topA}$ (TopA), $x_{rrn}$ (stable RNAs), $u_s$ (Signal).

P2 promoter region, rather than to the crp P1 promoter region (Gonzalez-Gil et al., 1998).

Different concentration thresholds of CRP are required in cells, to allow production of different levels of the cAMP CRP complex. Indeed, a low concentration of the complex is sufficient to stimulate crp expression in the presence of the carbon starvation signal. Above a second, higher threshold concentration, the complex...
controls the expression of a large set of genes involved in the adaptation network, up to the entry into stationary phase. Finally, above its highest threshold concentration, the complex inhibits its further accumulation, by shutting off its production through repression of Cya. This leads to three threshold concentrations of CRP, denoted by \( \theta_1^{\text{crp}}, \theta_2^{\text{crp}}, \) and \( \theta_3^{\text{crp}} \), which are ordered by the threshold inequalities

\[
0 < \theta_1^{\text{crp}} < \theta_2^{\text{crp}} < \theta_3^{\text{crp}} < \max_{\text{crp}}. \tag{18}
\]

By means of (17), we derive the possible synthesis and degradation rates of CRP, \( M_{\text{crp}} = [\kappa_1^{\text{crp}} + \kappa_2^{\text{crp}} + \kappa_3^{\text{crp}} + \kappa_4^{\text{crp}} + \kappa_5^{\text{crp}} + \kappa_6^{\text{crp}} + \kappa_7^{\text{crp}} + \kappa_8^{\text{crp}}$, and \( N_{\text{crp}} = \gamma_{\text{crp}} \), respectively. Because crp P1 is a house-keeping promoter (Gonzalez-Gil et al., 1998), we set \( \kappa_2^{\text{crp}}, \kappa_3^{\text{crp}}, \kappa_4^{\text{crp}}, \kappa_5^{\text{crp}}, \kappa_6^{\text{crp}}, \kappa_7^{\text{crp}}, \kappa_8^{\text{crp}}, \) and \( \kappa_9^{\text{crp}} > \theta_3^{\text{crp}} > \theta_2^{\text{crp}} > \theta_1^{\text{crp}} \). If this were not the case, CRP would not be able to reach a basal concentration at which it can stimulate its own expression when signal is switched on. The fact that crp P2 is a strong promoter (Gonzalez-Gil et al., 1998) implies that \( \kappa_2^{\text{crp}} + \kappa_3^{\text{crp}} > \theta_3^{\text{crp}} \), and \( \kappa_4^{\text{crp}} + \kappa_5^{\text{crp}} > \theta_3^{\text{crp}} \), because this allows CRP to reach a level at which regulation of cyA occurs. We thus arrive at the following equilibrium inequalities:

\[
\theta_1^{\text{crp}} < \theta_2^{\text{crp}} < \theta_3^{\text{crp}}, \quad \theta_1^{\text{crp}} + \kappa_2^{\text{crp}} < \theta_3^{\text{crp}}, \quad \theta_2^{\text{crp}} < \max_{\text{crp}},
\]

\[
\theta_1^{\text{crp}} < \theta_2^{\text{crp}} < \theta_3^{\text{crp}} + \kappa_4^{\text{crp}}, \quad \theta_2^{\text{crp}} < \max_{\text{crp}}.
\tag{19}
\]

Cya is present at low concentrations in the cell (Yang and Epstein, 1983). It is the product of the gene cyA, which is transcribed from three promoters: the constitutive P1 and P’1 promoters, and the regulated P2 promoter (Alb’s, 1985; Alb et al., 1983; Roy and Danchin, 1982). Control of expression of cyA is regulated by the complex cAMP-CRP, which represses gene transcription starting from the strong promoter P2 (Alb’s, 1985; Alb et al., 1983; Inada et al., 1986; Kawamukai et al., 1985; Mot and Alb, 1985). We use the rate constants \( \kappa_1^{\text{cyA}}, \kappa_2^{\text{cyA}}, \) and \( \kappa_3^{\text{cyA}}, \kappa_4^{\text{cyA}}, \kappa_5^{\text{cyA}}, \) to denote constitutive and cAMP-CRP-controlled synthesis of Cya. In the absence of information to the contrary, degradation of Cya is assumed not to be regulated and occurs with a rate constant \( \gamma_{\text{cyA}} \). Denoting by \( x_{\text{cyA}} \), the total concentration of Cya, we arrive at the following state equation:

\[
x_{\text{cyA}} = x_{\text{cyA}}(1 - x_{\text{cyA}}(\theta_1^{\text{cyA}} + \theta_2^{\text{cyA}} + \theta_3^{\text{cyA}}) x_{\text{cyA}}(\theta_1^{\text{cyA}} + \theta_2^{\text{cyA}})) x_{\text{cyA}} x_{\text{cyA}}
\tag{20}
\times s^3(\gamma_{\text{cyA}}, \theta_1^{\text{cyA}} - \gamma_{\text{cyA}} x_{\text{cyA}}).
\]

Because the complex cAMP-CRP exerts its regulatory functions at different thresholds in our model, following the reasoning of Section 4.2, we define the same number of thresholds for \( x_{\text{cyA}} \) as for \( x_{\text{crp}} \). We thus obtain the three thresholds \( \theta_1^{\text{cyA}}, \theta_2^{\text{cyA}}, \theta_3^{\text{cyA}} \) with the following inequalities:

\[
0 < \theta_1^{\text{cyA}} < \theta_2^{\text{cyA}} < \theta_3^{\text{cyA}} < \max_{\text{cyA}}. \tag{21}
\]

The possible synthesis and degradation rates of Cya can be deduced from (20). They are \( M_{\text{cyA}} = [\kappa_1^{\text{cyA}} + \kappa_2^{\text{cyA}} + \kappa_3^{\text{cyA}}] \) and \( N_{\text{cyA}} = \gamma_{\text{cyA}} \), respectively. P1 and P’1 are house-keeping promoters, allowing a constitutive expression of Cya during E. coli’s life cycle and hence \( \kappa_1^{\text{cyA}} = \kappa_2^{\text{cyA}} = \kappa_3^{\text{cyA}} > \theta_3^{\text{cyA}} \). Derepression of promoter P2 allows, together with constitutive expression from P1, maximal production of Cya. As a consequence, \( \kappa_1^{\text{cyA}} + \kappa_2^{\text{cyA}} + \kappa_3^{\text{cyA}} > \theta_3^{\text{cyA}} \). In summary, we have the following equilibrium inequalities:

\[
x_{\text{cyA}} < \frac{\kappa_1^{\text{cyA}} + \kappa_2^{\text{cyA}} + \kappa_3^{\text{cyA}}}{\gamma_{\text{cyA}}} < \theta_2^{\text{cyA}}, \quad \theta_2^{\text{cyA}} = \frac{\kappa_1^{\text{cyA}} + \kappa_2^{\text{cyA}} + \kappa_3^{\text{cyA}}}{\gamma_{\text{cyA}}} < \max_{\text{cyA}}. \tag{22}
\]
state equation for the protein

\[
x_{\text{fis}} = \frac{\kappa_{\text{fis}}}{\gamma_{\text{fis}}} (1 - \alpha_{\text{fis}} \rho_{\text{fis}} (\theta_{\text{fis}}, \theta_{\text{fis}}))
\times s^x (x_{\text{fis}}, \theta_{\text{fis}}) + \frac{\kappa_{\text{fis}}}{\gamma_{\text{fis}}} s^x (x_{\text{fis}}, \theta_{\text{fis}}) \times (x_{\text{fis}}, \theta_{\text{fis}})
\times s^x (x_{\text{fis}}, \theta_{\text{fis}}) X - s^x (x_{\text{fis}}, \theta_{\text{fis}}) s^x (x_{\text{fis}}, \theta_{\text{fis}})\]

(23)

Fis has five threshold concentrations, \(\theta_{\text{fis}}^0, \ldots, \theta_{\text{fis}}^5\). The first two of these correspond to concentration levels above which Fis represses the two cya promoters. \(\theta_{\text{fis}}^1\) corresponds to stimulation of rm production. Control of gyrAB and topoA promoters is exerted above the threshold \(\theta_{\text{fis}}^2\), whereas the protein represses its own expression above the threshold \(\theta_{\text{fis}}^3\). These five thresholds are ordered by the following inequalities:

\[0 < \theta_{\text{fis}}^0 < \theta_{\text{fis}}^1 < \theta_{\text{fis}}^2 < \theta_{\text{fis}}^3 < \max_{\text{fis}}.\]  

(24)

The possible synthesis and degradation rates are given by \(M_{\text{fis}} = [x_{\text{fis}}^0, x_{\text{fis}}^1, x_{\text{fis}}^2] + N_{\text{fis}} = [\gamma_{\text{fis}} \times 3]\), respectively. We have to order the target equilibrium values \(x_{\text{fis}}^0, x_{\text{fis}}^1, x_{\text{fis}}^2, x_{\text{fis}}^3\) and \(x_{\text{fis}}^4, x_{\text{fis}}^5\) with respect to the threshold values. From the very beginning of exponential phase, the Fis concentration should always be high enough to repress the strong promoter \(\rho_{\text{fis}}\). In order to maintain CRP concentration at a low level, this gives \(x_{\text{fis}}^0 \geq \gamma_{\text{fis}} \times 3\). Making the reasonable assumption that the Fis concentration may reach a level at which the negative feedback loop becomes active (23) implies \(x_{\text{fis}}^0 + x_{\text{fis}}^1 + x_{\text{fis}}^2 > \gamma_{\text{fis}} \times 3\). We thus have the following equilibrium inequalities for Fis:

\[\theta_{\text{fis}}^0 < \frac{x_{\text{fis}}^0}{\gamma_{\text{fis}}} < \theta_{\text{fis}}^1 < \frac{x_{\text{fis}}^1 + x_{\text{fis}}^2}{\gamma_{\text{fis}}} < \theta_{\text{fis}}^2 < \max_{\text{fis}}.\]  

(25)

5.3. DNA topology module

The DNA topology module contains genes encoding GyrAB, TopA, and Fis. These three proteins are involved in the homeostatic control of DNA topology in E. coli cells (Jensen et al., 1999; Menzel and Gellert, 1983; Schneider et al., 2000; Snoep et al., 2002; Travers et al., 2001). They ensure a fine-tuned control of the DNA supercoiling level, except during growth transitions, when DNA topology changes, for example as glucose becomes depleted in the growth medium (Balke and Gralla, 1987). As explained in Section 2, we consider the GyrAB complex as the product of a single gene gyrAB. This gene is transcribed from a unique promoter, whose activity is regulated by DNA supercoiling and the protein Fis (Adachi et al., 1984; Menzel and Gellert, 1983, 1987a,b). We therefore consider that the protein is expressed at a synthesis rate \(k_{\text{gyrAB}}\) from this promoter and is degraded at a rate \(\gamma_{\text{gyrAB}}\). We then obtain the following state equation for GyrAB:

\[\dot{x}_{\text{gyrAB}} = k_{\text{gyrAB}} (1 - s^x (x_{\text{gyrAB}}, \theta_{\text{gyrAB}})) \times s^x (x_{\text{gyrAB}}, \theta_{\text{gyrAB}})\]

(26)

In order to allow DNA topology to attain the level at which autoregulation sets in, GyrAB should reach its maximal concentration threshold, that is, \(k_{\text{gyrAB}} / \gamma_{\text{gyrAB}} > \theta_{\text{gyrAB}}\). We thus obtain the following equilibrium inequalities:

\[\theta_{\text{gyrAB}} < \frac{k_{\text{gyrAB}}}{\gamma_{\text{gyrAB}}} \times \max_{\text{gyrAB}}.\]  

(27)

The gene topoA is transcribed from five promoters, whose exact contribution to the TopA expression level is not yet well-understood (Qi et al., 1997; Tse-Dinh and Beran, 1988). They most likely allow expression of the topoisomerase under a wide range of environmental conditions. For the sake of simplicity, we consider topoA here as being expressed from a single promoter. The expression of this gene is also controlled by Fis and DNA supercoiling, but in comparison with gyrAB in the opposite way: topoA is activated by a low level of DNA supercoiling and by Fis (Travers et al., 2001; Weinstein-Fischer et al., 2000). Considering that TopA is synthesized at a rate \(k_{\text{topA}}\) and degraded at a rate \(\gamma_{\text{topA}}\), we arrive at the following state equation:

\[\dot{x}_{\text{topA}} = k_{\text{topA}} s^x (x_{\text{gyrAB}}, \theta_{\text{gyrAB}}) \times s^x (x_{\text{topA}}, \theta_{\text{topA}})\]

From Eq. (29) we can deduce the possible synthesis and degradation rates: \(M_{\text{topA}} = [\max_{\text{topA}}]\) and \(N_{\text{topA}} = [\gamma_{\text{topA}}\times 5].\)
\[ \gamma_{\text{topA}} \text{, respectively. As for GyrAB, we have to distinguish two different threshold concentrations for TopA, } \theta_{\text{topA}}^1 \text{ and } \theta_{\text{topA}}^2 \text{, which are ordered in the following manner:} \\
0 < \theta_{\text{topA}}^1 < \theta_{\text{topA}}^2 < \max_{\text{topA}}. \]  

(30)

Stimulation of the topA promoter by DNA supercoiling and Fis allows maximal production of TopA. We therefore set \( \kappa_{\text{topA}}/\gamma_{\text{topA}} > \theta_{\text{topA}}^2 \). This motivates the following inequalities:

\[ \theta_{\text{topA}}^2 < \kappa_{\text{topA}}/\gamma_{\text{topA}} < \max_{\text{topA}}. \]  

(31)

5.4. Stable RNA output module

In E. coli, the production of stable RNAs varies in proportion to the growth rate, to match the cell’s changing demand for protein synthesis. Most of this regulation is achieved at the level of transcription of the seven rrn operons (Dennis et al., 2004; Murray et al., 2003; Murray and Gourse, 2004; Paul et al., 2004; Schneider et al., 2003). Stable RNAs are produced from two promoters, P1 and P2. The activity of the former is stimulated by the protein Fis, which allows rapid bacterial growth (Appleman et al., 1998; Nilsson et al., 1992; Zhang and Bremer, 1996; Zhang et al., 2002). On the contrary, rrn P2 is considered a house-keeping promoter, because it is much less responsive (Schneider et al., 2003) and no Fis-dependent regulation has been demonstrated. We consider that stable RNAs are produced from rrn P1 at a synthesis rate \( \dot{e}_{\text{rrn}} \), and from rrn P2 at a rate \( \dot{e}_{\text{rrn}}^2 \). Assuming these RNAs are degraded at a rate \( \gamma_{\text{rrn}} \), we obtain the following state equation for stable RNAs:

\[ \dot{x}_{\text{rrn}} = \dot{e}_{\text{rrn}} \dot{s}^+ (x_{\text{Fis}}, \theta_{\text{Fis}}^1) + \dot{e}_{\text{rrn}}^2 - \gamma_{\text{rrn}} x_{\text{rrn}}. \]  

(32)

Analysis of Eq. (32) shows that there are two possible synthesis rates for stable RNAs, \( M_{\text{rrn}} = (\dot{e}_{\text{rrn}}^1 + \dot{e}_{\text{rrn}}^2)/\gamma_{\text{rrn}} \), and one degradation rate, \( N_{\text{rrn}} = \gamma_{\text{rrn}} \). We distinguish a single threshold, \( \theta_{\text{rrn}} \), above which the stable RNA concentration is assumed high enough to allow bacterial growth. This leads to the straightforward threshold inequalities:

\[ 0 < \theta_{\text{rrn}} < \max_{\text{rrn}}. \]  

(33)

We now have to order the target equilibrium values \( \dot{e}_{\text{rrn}}^1/\gamma_{\text{rrn}} \) and \( \dot{e}_{\text{rrn}}^2/\gamma_{\text{rrn}} \) with respect to the threshold values of \( x_{\text{rrn}} \). The increase of the production of stable RNAs during exponential phase, due to the activation by Fis, implies that \( \dot{e}_{\text{rrn}} + \dot{e}_{\text{rrn}}^2 > \gamma_{\text{rrn}} \). In stationary phase, these RNAs are no longer produced, which gives \( \dot{e}_{\text{rrn}}/\gamma_{\text{rrn}} < \theta_{\text{rrn}} \). In summary, we have the following equilibrium inequalities:

\[ 0 < \frac{\dot{e}_{\text{rrn}}^1}{\gamma_{\text{rrn}}} < \theta_{\text{rrn}}, \quad \theta_{\text{rrn}} < \frac{\dot{e}_{\text{rrn}}^2 + \dot{e}_{\text{rrn}}^1}{\gamma_{\text{rrn}}} < \max_{\text{rrn}}. \]  

(34)

6. Simulation of carbon starvation response

The model presented in Section 5 consists of six state variables, one input variable, 17 threshold parameters, and 17 rate parameters. In addition, we have specified 48 threshold and equilibrium inequalities. The choice for particular regulation functions and parameters inequalities was motivated by the available genetic and molecular data on the carbon starvation response in E. coli. Using the model, we simulate in this section the response of the bacterium to the depletion or sudden availability of an essential carbon source in the growth medium. The simulations lead to qualitative behavioral predictions that can be compared with experimental observations reported in the literature. All simulations described below have been carried out by means of the computer tool GNA (Section 3).

In Section 6.1, we simulate the transition of E. coli from exponential to stationary phase in response to a carbon deprivation. In agreement with biological data, the propagation of the carbon starvation signal through the network leads to the adaptation of cellular growth. However, the DNA supercoiling level is not adjusted, which is inconsistent with published data and suggests new experiments. In Section 6.2, we simulate the transition of E. coli from stationary to exponential phase in response to a carbon upshift. Bacterial growth indeed adapts to the sudden availability of the carbon source, but does so in an unexpected way, through damped oscillations towards a new equilibrium value of the concentrations of some of the network components. Interestingly, this observation has never been reported in the literature. A close examination of the nutritional stress response network gives an explanation of how the damped oscillations emerge from the interactions between the network components.

6.1. Simulation of the entry into stationary phase

E. coli cells grow exponentially in a fresh growth medium, until an essential nutrient is depleted, obliging cells to slow down their growth and enter stationary phase. The adaptation of the cells to the changing nutrient conditions relies on the intricate network of interactions between the global regulators of the bacterium (Fig. 2). The carbon starvation signal acts on this network through the activation of the adenylate cyclase, Cya, and the subsequent formation of the cAMP-CRP complex.
In the absence of the carbon starvation signal \( (0 \leq u_s < \theta_s) \), the system has a single qualitative equilibrium state corresponding to the following switching domain:

\[
\begin{align*}
\theta_{crp}^2 < x_{crp} &< \theta_{crp}^3, \\
x_{fu} &< \theta_{fu}, \\
x_{gyrAB} &< \theta_{gyrAB}, \\
0 \leq x_{topA} &< \theta_{topA}, \\
\theta_{rrn}^1 < x_{rrn} &< \theta_{rrn}, \\
0 \leq u_s &< \theta_s.
\end{align*}
\]

That is, the switching domain contains an equilibrium point located on the intersection of the threshold hyper-planes \( x_{fu} = \theta_{fu} \) and \( x_{gyrAB} = \theta_{gyrAB} \) (recall from Section 3.2 that the PL model can be analyzed in switching domains by extending the differential equations to differential inclusions).

The qualitative equilibrium state is consistent with physiological conditions found in exponentially-growing cells. In fact, in the course of the exponential phase, \( E. coli \) cells have been shown to contain a low concentration of CRP (Ishizuka et al., 1994), while Cya (Saier and Ramseier, 1996) and Fis (Ali Azam et al., 1999; Ball et al., 1992; Pratt et al., 1997) are at a high level. Even at its maximum level though, the concentration of Cya remains low in comparison with that of Fis. In addition, the DNA is negatively supercoiled at a high level, corresponding to GyrAB and TopA concentrations above which \( fu \) activation and gyrAB inhibition (and thus homeostatic regulation) sets in.

Suppose that the system is in the above qualitative equilibrium state and that we perturb the system by switching on the carbon starvation signal \( (\theta_s \leq u_s \leq \max_s) \). Simulation of the network from the resulting initial state takes less than one second to complete on a PC (2.4 GHz, 512 Mb) and gives rise to a transition graph of 65 qualitative states. Many of these states are associated with switching domains that the system traverses instantaneously. Since the biological relevance of the latter states is limited, they can be eliminated. This leads to a reduced transition graph of 26 qualitative states. The state transition graph contains a single qualitative equilibrium state that the system eventually reaches from all other states in the graph:

\[
\begin{align*}
\theta_{crp}^2 < x_{crp} &< \theta_{crp}^3, \\
x_{fu} &< \theta_{fu}, \\
x_{gyrAB} &< \theta_{gyrAB}, \\
0 \leq x_{topA} &< \theta_{topA}, \\
0 \leq x_{rrn} &< \theta_{rrn}, \\
0 \leq u_s &< \theta_s.
\end{align*}
\]

A typical qualitative behavior is shown in Fig. 13. The first event after receiving the carbon starvation signal is the decrease of the Fis concentration. As a consequence, the level of stable RNAs starts to decrease as well. The next event concerns the increase of the CRP concentration, followed by a decrease of the level of Cya. In parallel, the concentration of GyrAB increases, whereas the concentration of TopA remains below its first threshold, which causes the level of DNA supercoiling to rise. Note that \( x_{topA} < \theta_{topA} \) does not mean that the protein is absent, but rather that it has not reached a level above which it activates gyrAB expression and inhibits topA expression.

As explained in Section 2, the concentration of the stable RNAs is a good indicator of the cell’s growth state. Whereas cells in exponential phase need massive amounts of the RNAs for the translation of the proteins, a low concentration of the stable RNAs is sufficient for the reduced activity of the translation machinery in stationary phase (Bremer and Dennis, 1996; Keener and Nomura, 1996). Since the level of RNAs is low in the qualitative equilibrium state reached by the system after a carbon starvation signal, we conclude that this state is representative for a stationary-phase cell. The process driving the cell’s growth arrest can be explained by relating the qualitative behavior to the carbon starvation response network in Fig. 2. During exponential phase, the adenylate cyclase is present, but inactive. When the carbon starvation signal is switched on, it activates the protein and thus enables it to produce cAMP. The metabolite binds to CRP, which is not yet abundant, thus giving rise to a low concentration of the cAMP-CRP complex. The level of cAMP-CRP is nevertheless high enough to start repressing the expression of \( fu \). This stimulates further accumulation of CRP, and thus further repression of Fis, through derepression of the Fis-controlled promoters of cya. The decrease of the Fis concentration also causes the downregulation of the expression of the \( rrr \) genes. As a consequence, the level of the stable RNAs decreases and the cell enters stationary phase.

We conclude from our model of the carbon starvation response network that a positive feedback mechanism, the mutual inhibition of \( fu \) and \( cya \), plays a key role in the
Fig. 13. Temporal evolution of the protein and stable RNA concentrations in a typical qualitative behavior in the state transition graph, when signal is present ($\theta_s < \theta_s\leq \max_s$). The behavior represents the molecular events accompanying the transition from exponential to stationary phase following carbon starvation. Under carbon deprivation conditions, it causes a switch from a state in which Fis is high and CRP low to a state in which Fis is low and CRP high. The predicted evolution of the Fis concentration is in agreement with experimental data (Ali Azam et al., 1999; Ball et al., 1992; Pratt et al., 1997) showing a 50-fold decrease of the level of this protein when going from exponential to stationary phase. Unfortunately, similar measurements are not available for CRP, although available evidence tends to confirm the model predictions. Whereas the protein has been shown to accumulate to low concentrations in the presence of glucose,
that is, under conditions of exponential growth, a high concentration is observed when glucose is absent (which is the case under stationary-phase conditions (Ishizuka et al., 1994)).

The predicted qualitative evolution of the protein concentrations in Fig. 13 implies that the DNA supercoiling level increases at the onset of stationary phase. In fact, the production of the cAMP CRP complex in response to the carbon starvation signal causes the concentration of Fis to decrease, as explained above. As a consequence, the protein can no longer repress the expression of GyrAB, whose concentration starts to increase. Given that the concentration of TopA remains low, this means that the DNA supercoiling level increases. However, this is not what has been observed experimentally. On the contrary, the DNA supercoiling level has been shown to decrease when E. coli cells enter stationary phase (Balke and Gralla, 1987). The inconsistency between the predicted and observed level of supercoiling suggests that our picture of the carbon starvation response network is incomplete, in the sense that interactions between the global regulators in Fig. 2, or additional regulators not shown in the figure, are missing. In Section 7, we propose experiments and model extensions to further investigate these possibilities.

6.2. Simulation of the reentry into exponential phase

When cells in stationary phase are put into fresh medium, the consequent nutrient upshift causes them to reenter exponential phase. Information on the sudden availability of a carbon source is transmitted to the carbon starvation response network through the deactivation of the adenylate cyclase, Cya, which stops formation of the CRP complex in response to the carbon starvation signal. Whereas the concentrations of CRP, Cya, and TopA remain constant along the cycle, the concentrations of Fis, GyrAB, and the stable RNAs oscillate. In particular, the GyrAB level starts to decrease, followed by the decrease of the level of Fis and, consequently, of the level of stable RNAs. The level of GyrAB then rises again, followed by increases of the levels of Fis and stable RNAs, which completes the qualitative cycle.

The information in the state transition graph is not sufficient to decide whether the qualitative cycle in Fig. 14 corresponds to a limit cycle or to trajectories spiraling towards an equilibrium point. However, a more detailed analysis (J.-L. Gouzé, personal communication) has shown that, for all parameters values satisfying the inequality constraints, the qualitative cycle corresponds to the second possibility, that is, to a damped oscillation towards an equilibrium point. The latter equilibrium point is located in the switching domain (35), associated with a qualitative equilibrium state in the transition graph. As explained in Section 6.1, this qualitative equilibrium state is representative for the physiology of an E. coli cell in exponential phase. That is, CRP is present in the cell at a low concentration, while the concentration of Cya is maximal and the concentration of Fis and the DNA supercoiling level are high. Notice that this state was used as the initial state for the simulation of the transition from exponential to stationary phase, after switching on the carbon starvation signal.

The occurrence of damped oscillations in response to a carbon upshift is an unexpected result of the simulation. However, the oscillations can be easily explained by looking at the regulatory interactions between Fis, GyrAB, and TopA in Fig. 2. The deactivation of Cya, following the switching-off of the carbon starvation signal, stops the production of cAMP CRP and leads to a reversal of the rates of the CRP and Fis concentrations: Fis starts to accumulate, whereas CRP disappears. The increase of the Fis concentration activates the expression of topA and represses the expression of gyrAB, thereby

\[ \frac{d\theta}{dt} < \theta_{\text{topA}} < \text{max}_{\theta_{\text{topA}}}, \]

\[ 0 \leq x_{\text{CRP}} < \theta_{\text{CRP}}, \]

\[ 0 \leq x_{\text{gyrAB}} < \theta_{\text{gyrAB}}, \]

\[ 0 \leq x_{\text{TopA}} < \theta_{\text{TopA}}, \]

\[ \theta_{\text{CTPR}} < \theta_{\text{topA}} < \text{max}_{\theta_{\text{topA}}}, \]

Given this state, we perturb the system by switching off the carbon starvation signal (0 < \theta_{\text{topA}} < \theta_{\text{topA}}). Simulation of the network from the resulting initial state takes less than one second to complete and gives rise to a transition graph of 429 qualitative states. Elimination of the instantaneous states leads to a reduced transition graph of 75 qualitative states. Unexpectedly, from all states in the graph the system eventually reaches a qualitative cycle.

A typical qualitative behavior leading to this qualitative cycle is shown in Fig. 14. After the carbon starvation signal is switched off, an increase of the Fis concentration is predicted, followed by a decrease of the CRP concentration, the exact opposite of what is seen in Fig. 13. The system then enters the qualitative cycle. Whereas the concentrations of CRP, Cya, and TopA remain constant along the cycle, the concentrations of Fis, GyrAB, and the stable RNAs oscillate. In particular, the GyrAB level starts to decrease, followed by the decrease of the level of Fis and, consequently, of the level of stable RNAs. The level of GyrAB then rises again, followed by increases of the levels of Fis and stable RNAs, which completes the qualitative cycle.

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Fig. 14. Temporal evolution of the protein and stable RNA concentrations in a typical qualitative behavior in the state transition graph, when Signal is absent (0 ≤ m_U < θ_S). The behavior represents the molecular events accompanying the transition from stationary to exponential phase following a carbon upshift.

lowering the DNA supercoiling level. This causes fis transcription from the P promoter to slow down, which restores GyrAB expression and inhibits TopA expression, thus increasing the negative supercoiling again. As a consequence, fis expression is stimulated, thus finishing the first period of the Fis-supercoiling oscillations.

Due to the regulation of the rrn genes by Fis, the concentration of the stable RNAs oscillates towards a new equilibrium level as well.

The predicted damped oscillations of the concentrations of Fis and GyrAB after a carbon upshift arise from the negative feedback loops relating these proteins and TopA. Currently, no experimental data are available to confirm or disconfirm the transient oscillations of the components of this homeostatic control mechanism.

7. Discussion

In this report, we have modeled and simulated the genetic regulatory network controlling the carbon starvation response in E. coli. We have first defined the carbon starvation response network by identifying key global regulators involved in the process (Section 2). Since quantitative information on the values of the kinetic parameters and molecular concentrations is lacking in most cases, we have chosen a qualitative modeling and simulation method to analyze the network. This method is based on a class of PL differential equations that use step functions in order to describe the regulatory mechanisms (Section 3). The step functions are approximations of sigmoidal functions accounting for complex
regulatory mechanisms involved in gene regulation, for instance, the effects of the activation of CRP and of DNA supercoiling on transcription initiation (Section 4). By integrating the available experimental data on the regulatory mechanisms underlying the interactions, we have developed a model of six piecewise-linear differential equations describing the carbon starvation response network (Section 5). Instead of assigning numerical values to the kinetic parameters, about fifty constraints in the form of algebraic inequalities have been obtained from the experimental literature.

Using this model, we have qualitatively simulated the carbon starvation response of *E. coli* cells by means of the computer tool GNA, and the simulation results have been compared with experimental data. The model predictions have led to new insights into how *E. coli* performs its growth transitions in response to changes in the nutrient conditions (Section 6) and has motivated concrete, clearly-defined experiments. In particular, the model suggests that the mutual inhibition of Fis and CRP plays a determining role in the transition from exponential to stationary phase. It also predicts that, in response to a carbon upshift, the concentrations of the key proteins Fis and GyrAB display damped oscillations towards a new equilibrium, representative of exponential-phase conditions. This surprising behavior, which has not been experimentally verified thus far, is a direct consequence of the homeostatic control of the DNA supercoiling level.

The mutual inhibition of Fis and CRP is a paradigm case of positive feedback, a control mechanism known to play a key role in developmental processes (Thomas and d’Ari, 1990). In response to a perturbation, it may cause the system to switch from one equilibrium to another, for instance, from a state with a high Fis concentration and a low CRP concentration to a state with a low Fis concentration and a high CRP concentration. Mechanisms similar to the mutual inhibition of Fis and CRP have been described for other bacterial stress responses. The best-known example is probably that consisting of the genes *cl* and *cro* of bacteriophage λ (Paulson, 1992), which are involved in the switch between the lytic and lysogenic states of infected *E. coli* cells.

To date no direct experimental data are available to verify the predicted occurrence of damped oscillations in the concentrations of the components Fis and GyrAB after a carbon upshift. In order to make up for this lack of data, we are actually carrying out in our laboratory measurements of the temporal evolution of the concentration of the global regulators in the carbon starvation response network. Despite the current absence of convincing evidence for the biological reality of the phenomenon, we might speculate on the function of these damped oscillations. In fact, the predicted oscillations arise from the negative feedback loop involving Fis and the proteins regulating the DNA supercoiling level. In a recent article, Rosenfeld et al. have argued that negative feedback loops may speed up cellular response times (Rosenfeld et al., 2002). It is obvious that a rapid adaptation of the cell to the sudden availability of a carbon source may confer a decisive growth advantage to *E. coli* cells.

Our model of the carbon-starvation response is a simplified representation of reality. While it provides a good explanation of how the adaptation of cellular growth to carbon availability emerges from the interactions between the global regulators of the bacterium, it does not give a complete picture. One of the aspects that has been omitted for simplicity is that the gradual slowing-down of the bacterial growth rate during the transition from exponential to stationary phase influences the value of the degradation parameters. In fact, these values decrease with the growth rate, since dilution of cellular components due to growth is their major determinant. In order to verify that omitting this aspect does not affect the conclusions of our study, we have built a more complex model including growth-regulated degradation terms. For instance, in the state equation of TopA the term $\gamma_{topA} x_{topA}$ has been replaced by $\gamma_{topA} x_{topA} + \gamma_{topA} x_{rrn} x_{rrn}$. Here, $\gamma_{topA}$ denotes the specific degradation rate of TopA in stationary phase and $\gamma_{topA} x_{rrn} x_{rrn}$ the rate at which TopA disappears in exponential phase, due to specific degradation and growth dilution. The equilibrium inequalities have been adapted accordingly and all qualitative simulations have been repeated with this more complex model. We have found that the size of the state transition graph slightly changes in some simulations, but that the predicted qualitative properties of the dynamics are preserved.

Certain predictions of the model are inconsistent with the biological observations, as noted in Section 6.1. This is for instance the case for the evolution of the DNA supercoiling level during the transition from exponential to stationary phase. Whereas our model predicts an increase of the DNA supercoiling level in response to the accumulation of GyrAB, the opposite has been experimentally observed (Balke and Gralla, 1987). Another discrepancy arises when simulating mutants of the genes *crp* or *cya*. While the model correctly predicts that mutation or deletion of these genes impairs the carbon starvation response, in the sense that it affects the bacterial growth transitions and the catabolic repression, it does not account for the slow-growth properties exhibited by *crp* strains (Kumar, 1976).
The above inconsistencies between biological observations and model predictions point to the limits of the model of the carbon starvation response network, but, more importantly, suggest its possible extensions. Several possible modifications of our current model could be imagined in order to reconcile the predicted and observed changes in the DNA supercoiling level during growth transitions. One way to extend the model is to include global regulators, currently missing from Fig. 2 in the model. For instance, by including the sigma factor RpoS, responsible for the general stress response in *E. coli* (Hengge-Aronis, 1996, 2000, 2002; Wick and Egli, 2004) in the model, we are able to prevent an increase of the DNA supercoiling level during the transition to stationary phase (results not shown). This model extension poses several interesting questions on the relative role of the different global regulators in the control of the DNA topology, questions that we are currently investigating.

This example brings to the fore that the carbon starvation response network is part of a larger genetic regulatory network comprising other global regulators such as RpoS, ppGpp, IHF, FNR, Lrp, HNS, and ArcA, in addition to DNA supercoiling, cAMP·CRP, and Fis (Madan Babu and Teichmann, 2003; Hengge-Aronis, 1996, 2000; Martinez-Antonio and Collado-Vides, 2003; Shen-Orr et al., 2002). This network senses and responds to a variety of stresses acting, often simultaneously, on the bacteria. Our ultimate goal is to model the whole network of global regulators to understand how it coordinates the different stress responses and allows *E. coli* to adapt its lifestyle to a wide range of environmental conditions.

**Acknowledgments**

We acknowledge the financial support of the ARC initiative of INRIA (project GDyn), the ACI IMP-Bio initiative of the French Ministry for Research (project BacAttract), and the NEST Adventure programme of the European Commission (project Hygeia). We are grateful to Julio Collado-Vides and the referees for comments on an earlier version of this article.

**Appendix A. Kinetic models of regulatory mechanisms**

In this section, we present the kinetic models that were developed to describe the control of gene expression by a transcriptional regulator modified through a signal-transduction pathway (Appendix A.2), and by the DNA supercoiling level (Appendix A.3).

### A.1. Transcriptional regulator

Consider the regulation of *rrn* by Fis in Fig. 5(a). Using the mass-action rate law, a detailed kinetic model of this mechanism could be developed, by taking into account the cooperative interaction of Fis and RNA polymerase, and the presence of multiple Fis-binding sites. For our purpose, it is sufficient to use the Hill rate law to describe the regulation. This law states a simple phenomenological relation between the activity of a gene and the concentration of a transcriptional regulator (Glass and Kauffman, 1973; Heinrich and Schuster, 1996). Denoting by $\alpha_{rrn}$ the normalized activity of gene *fis*, $0 \leq \alpha_{rrn} \leq 1$, and by $x_{fis}$ the concentration of Fis, the Hill function is defined as:

$$\alpha_{rrn} = \frac{x_{fis}}{K_{fis} + x_{fis}},$$

where $\sigma > 1$ is the cooperativity coefficient and $K_{fis}$ a phenomenological constant similar to the half-saturation constant in Michaelis-Menten kinetics. The Hill function has a sigmoidal shape, which corresponds to experimental observations of bacterial gene regulation (Ptashne, 1992; Yagil and Yagil, 1971).

Even though we ignore the precise values of the parameters, using reasonable estimates, we obtain the gene activity plots in Fig. 6 ($K_{fis} = 5 \cdot 10^{-5} \text{M}$ and $\sigma = 3$ in (a); $K_{fis} = 10^{-5} \text{M}$ and $\sigma = 3$ in (b)). All calculations and plots have been produced using the program Matlab (MathWorks).

### A.2. Transcriptional regulator activated through a signal-transduction pathway

Since cAMP·CRP stimulates transcription in a cooperative way (Joung et al., 1993), activation of the gene *crp* by the cAMP·CRP complex is described by means of the Hill rate law:

$$\alpha_{crp} = \frac{x_{crp\_comp}}{x_{crp\_comp} + K_{crp\_comp}},$$

where $x_{crp\_comp}$ and $\alpha_{crp}$ denote the concentration of cAMP·CRP and the normalized activity of *crp*, respectively. The constants $K_{crp\_comp}$ and $\sigma$ have their usual meaning.
The box ‘CRP activation’ in Fig. 7 involves the following reactions and rate constants:

\[
\begin{align*}
&\text{Cya}^* + \text{ATP} \rightleftharpoons \text{Cya}^* \cdot \text{ATP} \rightleftharpoons \text{Cya}^* \\
&\text{CAMP} \rightarrow \text{export/degradation},
\end{align*}
\]

where \( \text{Cya}^* \) corresponds to the activated form of the adenylate cyclase Cya, in the presence of signal. In addition to binding CRP, cAMP can be exported out of the cell or degraded at a rate \( k_d \), proportional to the cAMP concentration (Fraser and Yamazaki, 1979; Yang and Epstein, 1983). Within the cell, CRP monomers associate to form dimers with an affinity constant that is so high \( 10^9 - 10^{10} \text{M}^{-1} \) (Harman, 2001) that most likely the protein only exists in a dimeric state in the cell. This is the reason why we do not take into account the monomeric form of CRP here.

The reactions are modeled by means of the mass-action law. Let \( x_{\text{atp}}, x_{\text{camp}}, x_{\text{crp}}, x_{\text{cya}} \) denote the concentrations of free ATP, cAMP, CRP, and Cya, respectively. Then we obtain the following system of differential equations:

\[
\begin{align*}
\dot{x}_{\text{atp}} &= k_1 x_{\text{cya}} x_{\text{crp}} - k_2 x_{\text{cya}} x_{\text{atp}} - k_3 x_{\text{atp}} x_{\text{crp}} x_{\text{cya}} x_{\text{camp}} \quad (A.3) \\
\dot{x}_{\text{cya}} &= (k_{-1} + k_2) x_{\text{cya}} x_{\text{atp}} - k_1 x_{\text{cya}} x_{\text{atp}} x_{\text{crp}} x_{\text{cya}} x_{\text{camp}} \quad (A.4) \\
\dot{x}_{\text{crp}} &= k_1 x_{\text{cya}} x_{\text{atp}} x_{\text{crp}} x_{\text{camp}} - (k_{-2} + k_2) x_{\text{cya}} x_{\text{atp}} x_{\text{crp}} x_{\text{camp}} \quad (A.5) \\
\dot{x}_{\text{camp}} &= k_2 x_{\text{cya}} x_{\text{crp}} x_{\text{camp}} - k_{-4} x_{\text{crp}} x_{\text{camp}} \quad (A.6) \\
\dot{x}_{\text{crp}} &= k_{-4} x_{\text{crp}} x_{\text{camp}} - k_5 x_{\text{crp}} x_{\text{camp}} \quad (A.7) \\
\dot{x}_{\text{camp}} &= k_5 x_{\text{crp}} x_{\text{camp}} - k_{-4} x_{\text{crp}} x_{\text{camp}} \quad (A.8)
\end{align*}
\]

In addition, we define the following conservation relations:

\[
\begin{align*}
x_{\text{atp}} &= x_{\text{crp}} + x_{\text{camp}} \quad (A.9) \\
x_{\text{cya}} &= x_{\text{crp}} + x_{\text{camp}} \quad (A.10)
\end{align*}
\]

The conservation relations \((A.9)-(A.12)\) and the approximation \((A.13)\) allow the kinetic model \((A.3)-(A.8)\) to be reduced to the following two differential equations:

\[
\begin{align*}
x_{\text{camp}} &= x_{\text{camp}} + x_{\text{crp}} \quad (A.11) \\
x_{\text{crp}} &= x_{\text{crp}} + x_{\text{cya}} \quad (A.12)
\end{align*}
\]

where \( x_{\text{crp}}, x_{\text{cya}}, x_{\text{camp}} \), and \( x_{\text{atp}} \) denote the total cellular CRP, Cya, cAMP, and ATP concentrations, respectively. That is, the total concentration of the proteins and metabolites equals the sum of the free and complexed concentration.

It is reasonable to assume Michaelis–Menten kinetics for the cAMP-synthesis reaction steps. However, the affinity constant of the protein for ATP is below the intracellular ATP concentration under all growth conditions (Yang and Epstein, 1983), and the Michaelis–Menten rate law therefore simplifies to a pseudo-first-order rate law (see Chapter 3 in Fersht, 1985) and

\[
x_{\text{cya}} x_{\text{atp}} = K_a x_{\text{crp}} \quad (A.13)
\]

The above derivation is valid under the condition that the signal activating adenylate cyclase is present. In the absence of signal, the system of CRP activation is at equilibrium by definition. Since Cya synthesizes cAMP only in its activated form, we fix this equilibrium at \( x_{\text{crp}} = 0 \).

Based on the experimental literature, the following approximate parameter values have been chosen: \( k_1 = 100 \text{ s}^{-1} \) (Yang and Epstein, 1983), \( k_2 = 0.035 \text{ s}^{-1} \) (Epstein et al., 1975), \( K_a = 10^{-3} \text{ M} \) (Anderson et al., 1971), \( K_0 = 10^{-5} \text{ M} \) (Elbricht et al., 1989). For \( \sigma \), we have used the value 3.
as a function of the concentrations of CRP and Cya in the absence and presence of signal, leads to the plots shown in Fig. 9. The sigmoidal shape of the surface is robust under moderate variation of the parameter values. The activity plots for the regulation of fis are obtained in the same way, with a lower value for $K_a$ = $10^{-3}$ M.

A.3. DNA supercoiling

The regulation of fis in Fig. 10 can be modeled by means of an empirical model based on the DNA supercoiling level and the Hill rate law. The topoisomerases GyrAB and TopA ensure a fine-tuned control of the DNA supercoiling level by adding and removing negative supercoils, respectively (Hatfield and Benham, 2002). In the absence of more precise information, we describe these antagonistic influences on the DNA supercoiling level in steep sigmoid curves in the physiological range. We therefore define the normalized activity of the gene fis, $\alpha_{\text{fis}}$, by the following simple model:

$$ SC = a + b \frac{x_{\text{gyrAB}}}{x_{\text{topA}}} $$

(A.17)

where $x_{\text{gyrAB}}$ and $x_{\text{topA}}$ denote the topoisomerase concentrations, and $a$ and $b$ are real positive parameters.

The DNA supercoiling level acts as a transcriptional regulator, in the sense that it may activate or inhibit initiation of transcription (Hatfield and Benham, 2002). More precisely, measurements of the promoter activity as a function of the DNA supercoiling level have resulted in steep sigmoid curves in the physiological range. We therefore define the normalized activity of the gene fis, denoted by $\alpha_{\text{fis}}$, as depending on the DNA supercoiling level at a Hill rate law. We obtain the following equation:

$$ \alpha_{\text{fis}} = \frac{SC^m}{SC^m + K_{SC}} $$

(A.18)

where $\sigma$ is the cooperativity parameter, and $K_{SC}$ is a real constant analogous to the half-saturation constant in Michaelis-Menten kinetics. It describes the sensitivity of the fis promoter sequence to the DNA supercoiling level.

For arbitrary parameter values, we obtain the gene activity plots in Fig. 11 (a = 0.2, $b = 0.8$, $K_{SC} = 3$, and $\sigma = 4$ in (a); a = 0.1, b = 0.8, $K_{SC} = 3.5$, and $\sigma = 4$ in (b)). The results are robust, in the sense that moderate variations of the parameter values do not change the sigmoidal shape of the surfaces.

References


endoprotease glucose phosphotransferase system on the basis of kinetic measurements. J. Biol. Chem. 275 (45), 34941–34942.