Qualitative Modeling and Simulation of the Carbon Starvation Response in *E. coli*

Exercises

Delphine Ropers and Pedro Monteiro and Hidde de Jong
Institut National de Recherche en Informatique et en Automatique (INRIA)
Centre de recherche Grenoble - Rhône-Alpes
655 avenue de l'Europe, Montbonnot, 38334 Saint Ismier Cedex, France
{Delphine.Ropers,Pedro.Monteiro,Hidde.de-Jong}@inrialpes.fr

October 8, 2010

The common intestinal bacterium *Escherichia coli* is without any doubt the best-studied organism in biology. Apart from the fact that pathogenic strains of this bacterium cause, among other things, urinary infections in humans, it is important as a model organism for other bacteria and higher organisms. This has led the editors of a reference work on *E. coli* to remark that "all cell biologists have two cells of interest: the one they are studying and *Escherichia coli*" ([35], p. 4).

Upon depletion of an essential nutrient, like a carbon source, an exponentially-growing *E. coli* population may enter a non-growth state, making a transition from *exponential* to *stationary phase* (figure 1). During this growth-phase transition, individual bacteria develop a resistance to multiple stresses that allow them to survive the adverse environmental conditions. In this set of exercises, we will build mathematical models of the key part of the genetic regulatory network controlling the carbon starvation response of *E. coli* cells. The models will be used to simulate the transition from exponential to stationary phase by means of the program Genetic Network Analyzer (GNA).

After a brief introduction to the biology of the carbon starvation response in section 1, based on the description in [37], we propose a simple model of the network in section 2. The analysis of this model will give us an idea of how predictions on the qualitative behaviour of the system can be inferred from a formal representation of the interactions between molecular components of the cell. Section 4 will show how GNA can be used to simulate the carbon starvation response, using the simple model developed in the previous section. In section 5, GNA will be used to analyze a more complex model of the stress response network. In the final section, we will show how model-checking techniques can support this analysis.

1 Carbon starvation response in *E. coli*

On the molecular level, the transition from exponential to stationary phase in *E. coli* involves a variety of events [24, 26]. In particular, 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.

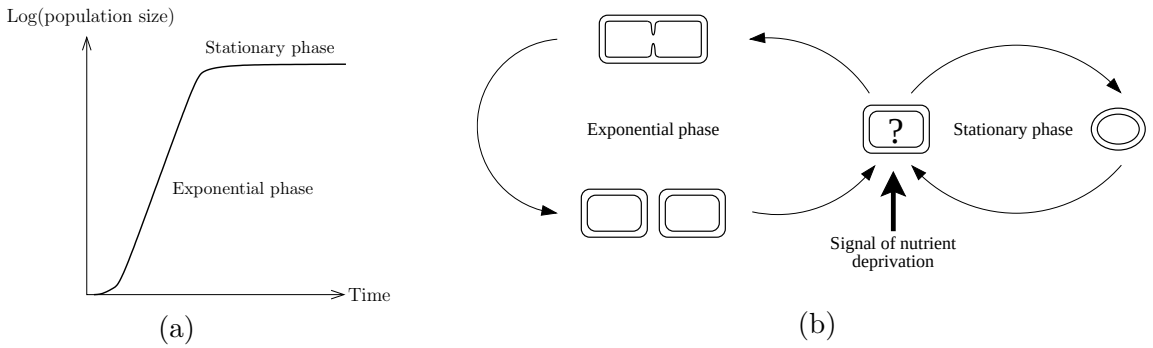

Figure 1: (a) Growth states of a bacterial population: exponential and stationary phase. (b) Nutrient-stress response of bacteria during the transition from exponential to stationary phase.

The morphological and physiological changes of *E. coli* cells that accompany the growth-phase transition are controlled at the genetic level by a complex regulatory network integrating various environmental signals [21, 25, 43]. Among the numerous genes, proteins, and metabolites making up this network, a class of pleiotropic transcription factors, called *global regulators*, plays a key role [19]. Global regulators mediate the activation or repression of a large set of genes in response to changes in environmental conditions, such as nutrient deprivation. Hence, they are able to regulate genes involved in a variety of cellular processes, such as different metabolic pathways. Many transcription factors have been identified in *E. coli*, but only a few of them are major global regulators [27, 31, 39].

How does the transition from exponential to stationary phase in response to a carbon starvation signal emerge from the network of global regulators and their interactions? Currently no clear answer to this question exists. However, it is known that the global regulators CRP and Fis play a key role in the control of the growth-phase transition. As a first approximation, we will therefore focus on a simple network composed of CRP and Fis, the genes encoding these proteins, and their mutual regulatory interactions.

The protein CRP is the major transcription regulator in *E. coli*, controlling the expression of hundreds of genes involved in the adaptative response of the cell to nutrient deprivation as well as in changes of cellular morphology and motility [22, 28]. The expression of the gene *crp* is repressed by the protein Fis. Additionally, Fis controls the expression of genes involved in the cellular metabolism [18]. For instance, it activates the *rrn* operons, which encode stable RNAs required for protein synthesis. The expression level the *rrn* operons is considered as representative of the cell's growth state. The expression of *fis* is controlled at the transcriptional level, where it is produced from a promoter repressed by CRP and Fis itself.

Simplifying a lot, we can say that the transition from exponential to stationary phase in response to a carbon starvation signal involves a switch from a state in which Fis is present at a high concentration and CRP at a low concentration to a state in which CRP is present at a high concentration and Fis at a low concentration. The low concentration of Fis in stationary phase causes the transcription of the stable RNA genes to be downregulated, entailing the growth arrest of the cell.

In order to understand how the switch from exponential to stationary phase emerges from the regulatory interactions between the genes *crp* and *fis*, and the proteins they encode, we

will build a simple mathematical model capturing essential aspects of the regulation of these genes.

2 Simple model of carbon starvation response network

As a preparatory step, the knowledge on the genes, proteins, and interactions is organized in a graphical representation of the regulatory network, as shown in figure 3. The representation uses symbols for protein synthesis and its activation and inhibition, following graphical conventions proposed in [30] (figure 2).

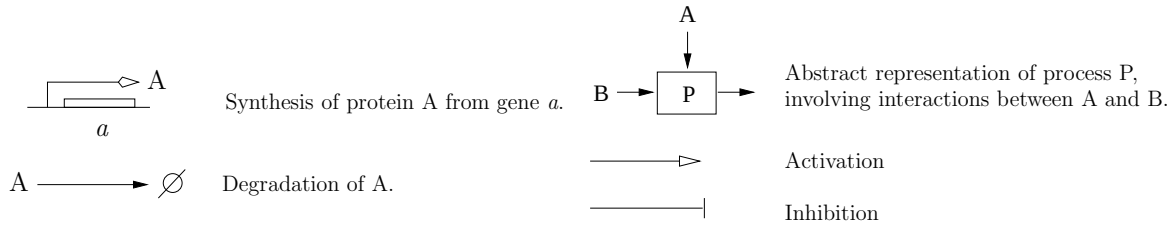

Figure 2: Notation for the graphical representation of genetic regulatory networks [30].

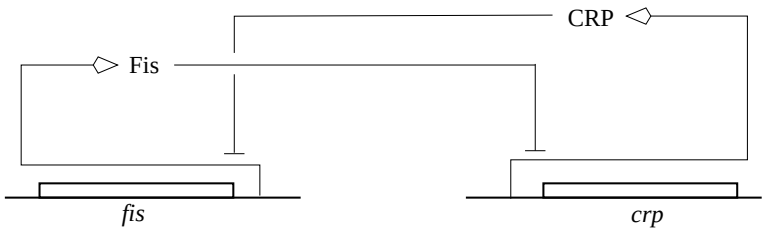

Figure 3: Graphical representation of the genetic regulatory network controlling the carbon starvation response in *E. coli* (see figure 2 for the notation). The network consists of the genes *crp* and *fis*, the proteins they encode, and their mutual regulatory interactions.

Next, the graphical representation of the network is transformed into a mathematical model. Particularly, we introduce non-negative concentration variables x_{CRP} and x_{Fis} for the proteins CRP and Fis, respectively. The rate of change of the concentrations can be described by means of the following pair of differential equations, expressing the difference of the rate of synthesis and the rate of degradation of the proteins:

$$\dot{x}_{CRP} = \kappa_{CRP}\, s^-(x_{Fis}, \theta_{Fis}) - \gamma_{CRP}\, x_{CRP}, \tag{1}$$

$$\dot{x}_{Fis} = \kappa_{Fis}\, s^-(x_{CRP}, \theta_{CRP}) - \gamma_{Fis}\, x_{Fis}, \tag{2}$$

where the *step functions* s^+, s^- are defined as follows:

$$s^+(x,\theta) = \begin{cases} 1, & x > \theta \\ 0, & x < \theta \end{cases} \qquad \text{and} \qquad s^-(x,\theta) = 1 - s^+(x,\theta). \tag{3}$$

The differential equation for CRP expresses that the protein is synthesized at a positive rate κ_{CRP}, if the concentration of Fis is below a threshold concentration θ_{Fis} ($\theta_{Fis} > 0$). In

that case, the step function $s^-(x_{Fis}, \theta_{Fis})$ evaluates to 1. However, if the concentration of Fis increases to above its threshold, the step function becomes 0 and the protein CRP is no longer synthesized. The step-function expression thus captures the inhibition of *crp* expression by Fis. CRP is degraded at a rate proportional to its own concentration, where γ_{CRP} denotes a degradation constant ($\gamma_{CRP} > 0$).

The regulation of *fis* expression involves CRP. If the CRP concentration is above the threshold θ_{CRP}, then the step-function expression $s^-(x_{CRP}, \theta_{CRP})$ equals 0 and *fis* expression is repressed. When the concentration of CRP is below its threshold, the step-function expression equals 1 and Fis is synthesized at a positive rate κ_{Fis}. As for CRP, the protein is degraded at a rate proportional to its own concentration ($\gamma_{Fis} > 0$).

The simple model (1)-(2) has two state variables, x_{CRP} and x_{Fis}, and can therefore be analyzed in the phase plane. As shown in figure 4, the phase plane is divided into four regions, called *regulatory domains*, by the threshold planes $x_{CRP} = \theta_{CRP}$ and $x_{Fis} = \theta_{Fis}$. The constants max_{CRP} and max_{Fis} denote maximum concentrations for CRP and Fis, respectively.

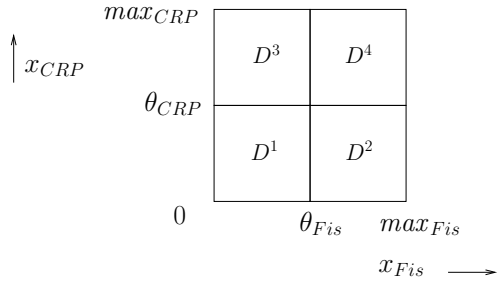

Figure 4: Phase plane and regulatory domains for the model (1)-(2) of the genetic regulatory network in figure 3.

Exercise 1 (Analysis of simple model in phase plane)
a. Show that in each regulatory domain the simple model reduces to two linear, uncoupled differential equations.
b. A differential equation of the form $\dot{x} = \kappa - \gamma\, x$ has a solution $x(t) = (\kappa/\gamma) - (\kappa/\gamma - x^0)\exp^{-\gamma t}$ on the time-interval $[0, \infty)$, where $x(0) = x^0$ represent the initial conditions. Write down the solution of the reduced model in each regulatory domain. What is the asymptotic behavior as $t \to \infty$?
c. Given that $\theta_{CRP} < \kappa_{CRP}/\gamma_{CRP} < max_{CRP}$ and $\theta_{Fis} < \kappa_{Fis}/\gamma_{Fis} < max_{Fis}$, sketch a few example solution trajectories in the regulatory domains.
d. Relate the qualitative dynamics of the system to the growth phases of the bacteria.

The exercise shows that global solutions of the model (1)-(2) can be obtained by piecing together local solutions in the regulatory domains. As you may have noticed in part c, it is not always possible to continue local solutions across threshold boundaries. This is for example the case for solution trajectories reaching the intersection of the threshold planes $x_{Fis} = \theta_{Fis}$ and $x_{CRP} = \theta_{CRP}$. In order to solve this problem, caused by the discontinuities in the right-hand side of the differential equations, we need to refine the analysis to the dynamics in *switching domains*, that is, regions located on the threshold boundaries. This requires sophisticated mathematical techniques that fall outside the scope of the exercises (but see [14, 20]).

The above mathematical analysis shows that we are able to make relevant predictions about the behavior of the system using qualitative information only. Instead of specifying numerical values for the threshold and rate parameters in (1)-(2), inequality constraints have been formulated. On one hand, the so-called *threshold inequalities* determine the partitioning of the phase plane into regulatory domains:

$$0 < \theta_{CRP} < max_{CRP}, \tag{4}$$

$$0 < \theta_{Fis} < max_{Fis}. \tag{5}$$

On the other hand, the *focal inequalities* fix the relative position of a regulatory domain and the *focal point* to which the trajectories in the domain converge:

$$\theta_{CRP} < \kappa_{CRP}/\gamma_{CRP} < max_{CRP}, \tag{6}$$

$$\theta_{Fis} < \kappa_{Fis}/\gamma_{Fis} < max_{Fis}. \tag{7}$$

The threshold and focal inequalities strongly constrain the local behavior of the system, while the global behavior can be inferred by piecing together the local solutions in the regulatory domains. Together, the differential equations and parameter inequalities form the *qualitative model* of the system.

3 Extended model of carbon starvation response network

The analysis of the simple cross-inhibition model of exercise 1 has shown the existence of two stable steady states, corresponding to the two growth phases of *E. coli*. What the model does not explain, however, is the switch from one steady state to the other, following carbon starvation. For this, we need to extend the model. In fact, it is known that in order to efficiently bind DNA, CRP has to be activated by the small metabolite cAMP, which is produced from ATP in response to a nutritional stress signal indicating the absence of carbon compounds such as glucose [28] (figure 5). In addition, CRP is expressed at a basal level in exponential phase, which is high enough to allow repression of *fis* expression when cAMP accumulates. How can the model be extended to include this information? This is the subject of the next exercise.

Exercise 2 (Analysis of extended model in phase plane)
a. Reformulate the simple model (1)-(2) so as to take into account the stress signal. Hint: define an input variable u_S representing the carbon starvation signal. If $s^+(u_S, \theta_S) = 1$, then the signal is present. Otherwise, it is absent.
b. Analyze the resulting model in the phase plane, following the steps of exercise 1. Distinguish between the cases in which the carbon starvation signal is present and in which it is absent.

4 Simulation of carbon starvation response using simple and extended models

The mathematical analysis explained above lies at the basis of a method for the qualitative simulation of genetic regulatory networks (see [2, 14] for a more general and technical descrip-

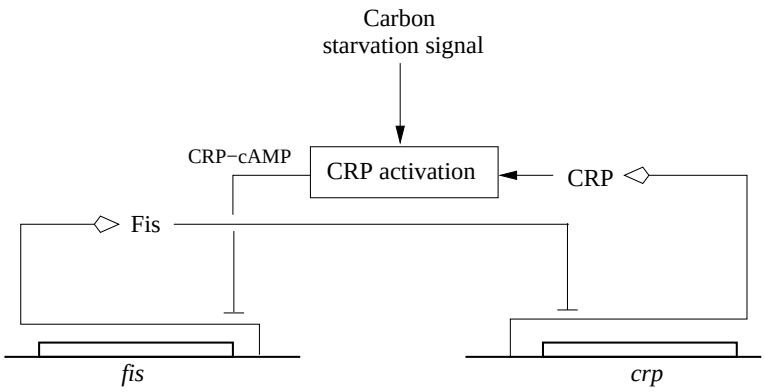

Figure 5: Graphical representation of the genetic regulatory network controlling the carbon starvation response in *E. coli* (see figure 2 for the notation). In addition to *crp* and *fis*, the proteins they encode, and their mutual regulatory interactions, the network includes the carbon starvation signal.

tion). The method has been implemented in Java in the computer program *Genetic Network Analyzer (GNA)* [13].

GNA allows the user to define and visually represent a genetic regulatory network, and build a qualitative model from this information by specifying for each gene the differential equations and inequality constraints. The model is analyzed by determining the steady states of the network and performing a so-called *qualitative simulation*. The simulation results in a state transition graph, consisting of domains and transitions between domains, starting from an initial domain defined by the user. GNA supports the visual analysis of the state transition graph by allowing the user, among other things, to zoom in or out from the graph, to reduce or expand the graph, to highlight qualitative states in the graph satisfying certain user-specified criteria, and to follow the qualitative evolution of the model variables along a path in the graph. Figure 6 shows a screenshot of GNA. A user manual of GNA can be obtained from the GNA web site.[1]

Exercise 3 (Simulation with simple and extended models.)
a. Start GNA with the command `gna`. Construct the simple model used in exercise 1 (store the model in the project `simpleModel.gnaml`). Similarly, enter initial conditions covering all possible values of CRP and Fis (call the initial conditions `simpleModel` and add them to the project).
b. Start a qualitative simulation with the model `simpleModel` and the initial conditions `simpleModel`.
c. Using GNA, verify the qualitative dynamics carried out in exercise 1.
d. Repeat the above steps for the extended model used in exercise 2. Call the project `extendedModel` and create two initial conditions, corresponding to stationary and exponential-phase conditions (`extendedModel1` and `extendedModel2`).

[1]GNA is distributed by the company Genostar and freely available for non-profit academic research purposes at `http://www-helix.inrialpes.fr/gna`.

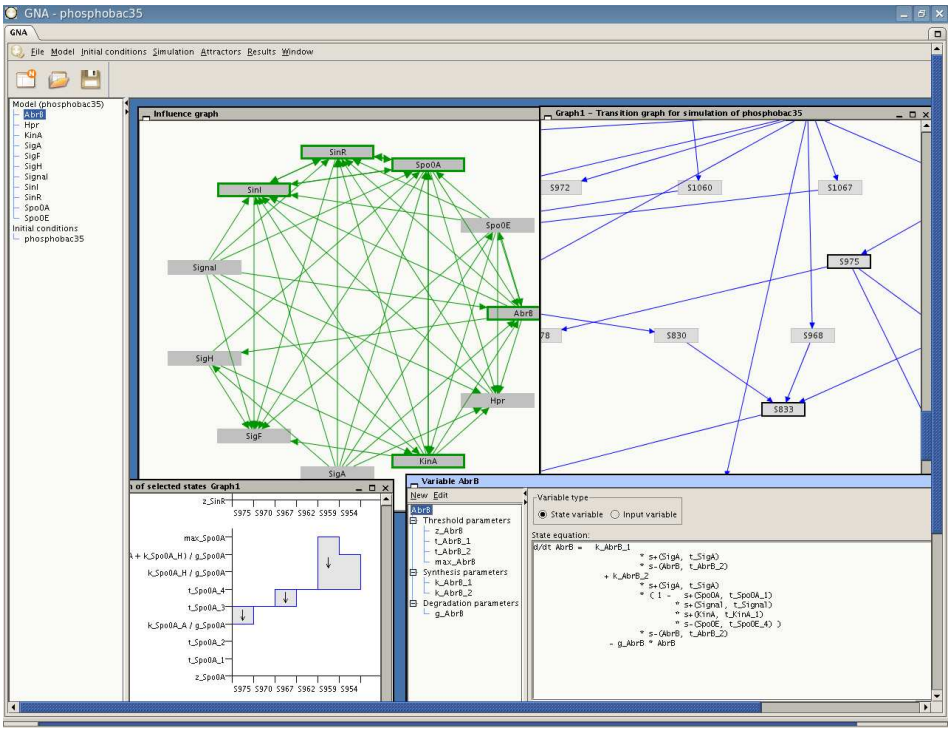

Figure 6: Screenshot of the qualitative modeling and simulation tool GNA.

5 Simulation of carbon starvation response using complex model

The models used in the previous sections are based on strongly-simplified versions of the carbon starvation response network. A picture that comes closer to the actual complexity of the network is shown in figure 7. It reveals that the regulation of the genes encoding CRP and Fis is much more complex than assumed above. For instance, the picture includes the carbon starvation signal activating CRP and the *rrn* genes encoding the stable RNAs. In addition, it takes into account the protein Cya, which is the enzyme catalyzing the production of cAMP from ATP, and the proteins TopA and GyrAB, which together control the DNA supercoling level, an important modulator of gene expression. The network is certainly not complete, but nevertheless allows an adequate description of some of the phenomena characterizing *E. coli* growth-phase transitions [36, 37].

Exercise 4 (Simulation with complex stress response model.)
a. Load the model of the carbon starvation response network in figure 7, stored in the project `ComplexModel.gnaml`, and compare the equations for Fis and CRP with those used in exercise 3.
b. Perform an attractor search and relate the steady states to exponential-phase and stationary-phase conditions. From these steady states, define appropriate initial conditions for carbon starvation and nutrient upshift. Call these initial conditions `complexModel1` and `complexModel2`, respectively, and start a qualitative simulation using the initial conditions `complexModel1`.
c. Using GNA, analyze the paths in the state transition graph representing the transition from exponential to stationary phase. Compare the results of the simulation with those obtained

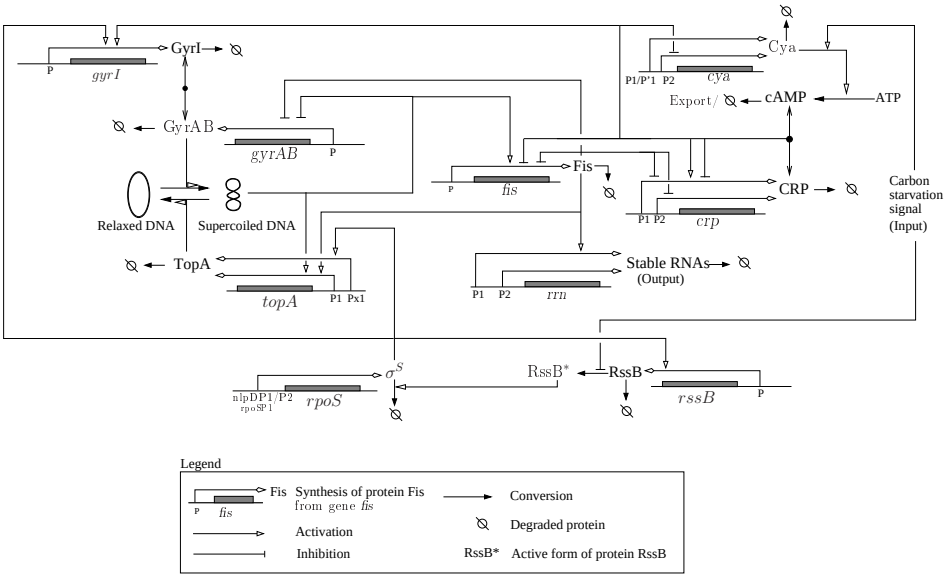

Figure 7: Network of key genes, proteins, and regulatory interactions involved in the carbon starvation response in *Escherichia coli* [36].

with the extended model in the previous exercise.

6 Automated verification of properties of carbon starvation model

The graphs obtained from the qualitative simulation of the carbon starvation network by means of `ComplexModel.gnaml` quickly become quite large, consisting of hundreds or thousands of states. As a consequence, it becomes almost impossible to gain an intuitive understanding of the role of a particular feedback mechanisms in the adaptation of the cell to nutritional stress conditions, such as the mutual inhibition of Fis and CRP, by checking thousands of paths in the graph by hand. The identification of interesting predictions from the model, concerning a specific mutant or physiological perturbation, is also compromised by the large size of the graphs.

Methods from the field of formal verification provide a promising way to deal with the analysis of large and complex models of genetic regulatory networks. Generally speaking, formal verification proceeds by specifying dynamical properties of interest as statements in temporal logic [9]. Efficient so-called *model-checking* algorithms exist to determine whether the statements are true or false, and thus whether the properties are satisfied by the model. The methods generally require the dynamics of the system to be described in the form of state transition graphs, like those used in GNA.

GNA has been extended with a model-checking functionality that allows the user to formulate properties in temporal logic and test these on a state transition graph, associated with a particular model and initial conditions [3, 34]. The actual verification of the property takes place through a web-service based connection of GNA with standard model checkers, like NuSMV [8, 33]. However, the user can also export the state transition graph to a text

file in a format that is accepted by these model checkers. The specification of the properties in temporal logic is supported by query patterns that capture the most-frequently asked questions by modelers.

Exercise 5 (Verification of properties of simple model.)
a. Start GNA with the command `gna`. Load the extended model used in exercise 3, stored in the project `ExtendedModel.gnaml`, and define initial conditions covering the entire state space. Call these initial conditions `extendedModel` and store them in the project.
b. Create atomic propositions corresponding to (i) a stable steady state, (ii) a low concentration of Fis and a high concentration of CRP, and (iii) carbon starvation (high stress signal). Call these atomic propositions `steadyState`, `highCRP_lowFis`, and `carbonStarvation`, respectively. Specify a property stating that it is possible to reach a stable steady state. Store this property in the project and test it with the model checker NuSMV from the initial conditions `extendedModel` (using implicit graphs).
c. Specify a property stating that if carbon starvation occurs, then the system necessarily reaches a stable steady state in which Fis is present a low concentration and CRP at a high concentration. Use the expert option of the property editor. Store this property in the project and test it with the model checker NuSMV (using implicit graphs).

The interest of the model-checking functionality will become especially clear from the next exercise, which deals with the entire model of the carbon starvation response network, based on figure 5.

Exercise 6 (Verification of properties of complex model.)
a. Load the model stored in the project `ComplexModel.gnaml`. Test the properties of the previous exercise on this model, after an appropriate reformulation of the atomic propositions and initial conditions (label these `complexModel1`). Use the model checker NuSMV and implicit graphs.
b. Create an atomic proposition named `decFis`, corresponding to a derivative of the concentration variable x_{Fis} that is negative (*i.e.*, $\dot{x}_{Fis} < 0$). Idem for `incCRP`, meaning $\dot{x}_{CRP} > 0$. Specify a property stating that an decreasing concentration of Fis is necessarily followed by an increasing concentration of CRP. Test this property with the initial conditions `complexModel1` and with initial conditions `complexModel` corresponding to all values of the concentration variables. Use the model checker NuSMV and implicit graphs.

7 Further reading

Several excellent and up-to-date textbooks on mathematical modeling of genetic and biochemical networks are available, such as [1, 5, 7, 16, 40, 42]. Reviews of methods for the modeling, simulation, and verification of genetic regulatory networks are [6, 10, 11, 17, 23, 29, 32, 38, 41]. More information on the qualitative simulation method used in these exercises can be found in [2, 12, 13, 14, 15] and at `http://ibis.inrialpes.fr/`, while the biology of the carbon starvation response in *E. coli* and the model used in sections 5 and 6 are explained in [36, 37] (see also [4, 21]).

References

[1] U. Alon. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman & Hall/CRC, Boca Raton, FL, 2007.

[2] G. Batt, H. de Jong, M. Page, and J. Geiselmann. Symbolic reachability analysis of genetic regulatory networks using discrete abstractions. *Automatica*, 44(4):982–989, 2008.

[3] G. Batt, D. Ropers, H. de Jong, J. Geiselmann, R. Mateescu, M. Page, and D. Schneider. Validation of qualitative models of genetic regulatory networks by model checking: Analysis of the nutritional stress response in *Escherichia coli*. *Bioinformatics*, 21(Suppl 1):i19–i28, 2005.

[4] K. Bettenbrock, S. Fischer, A. Kremling, K. Jahreis, T. Sauter, and E.D. Gilles. A quantitative approach to catabolite repression in *Escherichia coli*. *Journal of Biological Chemistry*, 281(5):2578–2584, 2005.

[5] H. Bolouri. *Computational Modeling of Gene Regulatory Networks – A Primer*. Imperial College Press, London, 2008.

[6] H. Bolouri and E.H. Davidson. Modeling transcriptional regulatory networks. *BioEssays*, 24(12):1118–1129, 2002.

[7] J.M. Bower and H. Bolouri, editors. *Computational Modeling of Genetic and Biochemical Networks*. MIT Press, Cambridge, MA, 2001.

[8] A. Cimatti, E. Clarke, E. Giunchiglia, F. Giunchiglia, M. Pistore, M. Roveri, R. Sebastiani, and A. Tacchella. NuSMV2: An OpenSource tool for symbolic model checking. In D. Brinksma and K.G. Larsen, editors, *14th International Conference on Computer Aided Verification (CAV 2002)*, volume 2404 of *Lecture Notes in Computer Science*, pages 359–364. Springer-Verlag, Berlin, 2002.

[9] E.M. Clarke, O. Grumberg, and D.A. Peled. *Model Checking*. MIT Press, Boston, MA, 1999.

[10] H. de Jong. Modeling and simulation of genetic regulatory systems: A literature review. *Journal of Computational Biology*, 9(1):67–103, 2002.

[11] H. de Jong and J. Geiselmann. Modélisation et simulation de réseaux de régulation génique par des équations différentielles ordinaires. In J.-F. Boulicaut and O. Gandrillon, editors, *Informatique pour l'analyse du transcriptome*, pages 143–185. Hermès, Paris, 2004.

[12] H. de Jong, J. Geiselmann, G. Batt, C. Hernandez, and M. Page. Qualitative simulation of the initiation of sporulation in *B. subtilis*. *Bulletin of Mathematical Biology*, 66(2):261–299, 2004.

[13] H. de Jong, J. Geiselmann, C. Hernandez, and M. Page. Genetic Network Analyzer: Qualitative simulation of genetic regulatory networks. *Bioinformatics*, 19(3):336–344, 2003.

[14] H. de Jong, J.-L. Gouzé, C. Hernandez, M. Page, T. Sari, and J. Geiselmann. Qualitative simulation of genetic regulatory networks using piecewise-linear models. *Bulletin of Mathematical Biology*, 66(2):301–340, 2004.

[15] H. de Jong and D. Ropers. Qualitative approaches towards the analysis of genetic regulatory networks. In Z. Szallasi, V. Periwal, and J. Stelling, editors, *System Modeling in Cellular Biology: From Concepts to Nuts and Bolts*, pages 125–148. MIT Press, Cambridge, MA, 2006.

[16] C.P. Fall, E.S. Marland, J.M. Wagner, and J.J. Tyson, editors. *Computational Cell Biology*. Springer-Verlag, New York, 2002.

[17] J. Fisher and T.A. Henzinger. Executable cell biology. *Nature Biotechnology*, 25(11):1239–1250, 2007.

[18] G. Gonzalez-Gil, P. Bringmann, and R. Kahmann. Fis is a regulator of metabolism in *Escherichia coli*. *Molecular Microbiology*, 22(1):21–29, 1996.

[19] S. Gottesman. Bacterial regulation: Global regulatory networks. *Annual Revue of Genetics*, 18(18):415–441, 1984.

[20] J.-L. Gouzé and T. Sari. A class of piecewise linear differential equations arising in biological models. *Dynamical Systems*, 17(4):299–316, 2002.

[21] R.M. Gutierrez-Ríos, J..A. Freyre-Gonzalez, O. Resendis, J. Collado-Vides, M. Saier, and G. Gosset. Identification of regulatory network topological units coordinating the genome-wide transcriptional response to glucose in *Escherichia coli*. *BMC Microbiology*, 7:53, 2007.

[22] J.G. Harman. Allosteric regulation of the cAMP receptor protein. *Biochimica et Biophysica Acta*, 1547(1):1–17, 2001.

[23] J. Hasty, D. McMillen, F. Isaacs, and J.J. Collins. Computational studies of gene regulatory networks: *In numero* molecular biology. *Nature Review Genetics*, 2(4):268–279, 2001.

[24] R. Hengge-Aronis. Regulation of gene expression during entry into stationary phase. In F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger, editors, *Escherichia coli and Salmonella: Cellular and Molecular Biology*, pages 1497–1512. ASM Press, Washington, DC, 2nd edition, 1996.

[25] R. Hengge-Aronis. The general stress response in *Escherichia coli*. In G. Storz and R. Hengge-Aronis, editors, *Bacterial Stress Responses*, pages 161–177. ASM Press, Washington, DC, 2000.

[26] G.W. Huisman, D.A. Siegele, M.M. Zambrano, and R. Kolter. Morphological and physiological changes during stationary phase. In F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger, editors, *Escherichia coli and Salmonella: Cellular and Molecular Biology*, pages 1672–1682. ASM Press, Washington, DC, 2nd edition, 1996.

[27] A. Ishihama. Prokaryotic genome regulation: multifactor promoters, multitarget regulators and hierarchic networks. *FEMS Microbiology Reviews*, 34(5):628–646, 2010.

[28] M.H. Saier Jr, T.M. Ramseier, and J. Reizer. Regulation of carbon utilization. In F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger, editors, *Escherichia coli and Salmonella: Cellular and Molecular Biology*, pages 1325–1343. ASM Press, Washington, DC, 2nd edition, 1996.

[29] G. Karlebach and R. Shamir. Modelling and analysis of gene regulatory networks. *Nature Reviews Molecular Cell Biology*, 9(10):770–780, 2008.

[30] K.W. Kohn. Molecular interaction maps as information organizers and simulation guides. *Chaos*, 11(1):84–97, 2001.

[31] A. Martinez-Antonio and J. Collado-Vides. Identifying global regulators in transcriptional regulatory networks in bacteria. *Current Opinion in Microbiology*, 6(5):482–489, 2003.

[32] H.H. McAdams and A. Arkin. Simulation of prokaryotic genetic circuits. *Annual Review of Biophysics and Biomolecular Structure*, 27:199–224, 1998.

[33] P.T. Monteiro, E. Dumas, B. Besson, R. Mateescu, M. Page, A.T. Freitas, and H. de Jong. A service-oriented architecture for integrating the modeling and formal verification of genetic regulatory networks. *BMC Bioinformatics*, 10:450, 2009.

[34] P.T. Monteiro, D. Ropers, R. Mateescu, A.T. Freitas, and H. de Jong. Temporal logic patterns for querying dynamic models of cellular interaction networks. *Bioinformatics*, 24(16):i227–i233, 2008.

[35] F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger, editors. *Escherichia coli and Salmonella: Cellular and Molecular Biology*. ASM Press, Washington, DC, 2nd edition, 1996.

[36] D. Ropers, V. Baldazzi, and H. de Jong. Model reduction using piecewise-linear approximations preserves dynamic properties of the carbon starvation response in *Escherichia coli*. *ACM/IEEE Transactions on Computational Biology and Bioinformatics*, 2009. In press.

[37] D. Ropers, H. de Jong, M. Page, D. Schneider, and J. Geiselmann. Qualitative simulation of the carbon starvation response in *Escherichia coli*. *Biosystems*, 84(2):124–152, 2006.

[38] T. Schlitt and A. Brazma. Current approaches to gene regulatory network modelling. *BMC Bioinformatics*, 8(Suppl. 6):S9, 2007.

[39] S.S. Shen-Orr, R. Milo, S. Mangan, and U. Alon. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nature Genetics*, 31(1):64–68, 2002.

[40] Z. Szallasi, V. Periwal, and J. Stelling, editors. *System Modeling in Cellular Biology: From Concepts to Nuts and Bolts*. MIT Press, Cambridge, MA, 2006.

[41] B. Di Ventura, C. Lemerle, K. Michalodimitrakis, and L. Serrano. From *in vivo* to *in silico* biology and back. *Nature*, 443(7111):527–523, 2006.

[42] E.O. Voit. *Computational Analysis of Biochemical Systems: A Practical Guide for Biochemists and Molecular Biologists*. Cambridge University Press, Cambridge, 2000.

[43] L.M. Wick and T. Egli. Molecular components of physiological stress responses in *Escherichia coli*. *Advances in Biochemical Engineering/Biotechnology*, 89:1–45, 2004.