INRIA

Team IBIS

Modeling, simulation, measurement, and control of bacterial regulatory networks

Grenoble - Rhône-Alpes

Theme : Computational Biology and Bioinformatics

Activity Report

2009

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2. Overall Objectives

2.1. Overview

Bacteria provide fascinating examples of the survival strategies developed by single-cell organisms to respond to environmental stresses. The stress responses of bacteria are controlled by large and complex networks of molecular interactions that involve genes, mRNAs, proteins, small effector molecules, and metabolites.

The study of bacterial stress response networks requires experimental tools for characterizing the interactions making up the networks and measuring the dynamics of cellular processes on the molecular level. In addition, when dealing with systems of this size and complexity, we need mathematical modeling and computer simulation to integrate available biological data, and understand and predict the dynamics of the system under various environmental and physiological conditions. The analysis of living systems through the combined application of experimental and computational methods has gathered momentum in recent years under the name of systems biology.

The first aim of the IBIS team is the unravelling of bacterial survival strategies through a systems-biology approach, making use of both models and experiments. In particular, we focus on the enterobacterium *Escherichia coli*, for which enormous amounts of genomic, genetic, biochemical and physiological data have been accumulated over the past decades. A better understanding of the survival strategies of *E. coli* in situations of nutritional stress is a necessary prerequisite for interfering with these strategies by specific perturbations or by even rewiring the underlying regulatory networks. This is the second and most ambitious aim of the project. It does not only spawn fundamental research on the control of living matter, but which may ultimately acquire medical relevance since *E. coli* serves as a model for many pathogenic bacteria.

The aims of IBIS raise four main challenges that generate new problems on the interface of (experimental) biology, applied mathematics, and computer science. In particular, the success of the project critically depends on (1) the modeling of large and complex bacterial regulatory networks, (2) the computer analysis and simulation of the network dynamics by means of these models, (3) high-precision and real-time measurements of gene expression to validate the models, and (4) the control and re-engineering of bacterial regulatory networks. While the first three items have been active research topics over the past few years, the control of regulatory networks is a novel challenge for IBIS that will be developed in the coming years.

The challenges of the research programme of the IBIS team require a wide range of competences on the interface of (experimental) biology, applied mathematics, and computer science. Since no single person can be expected to possess all of these competences, the international trend in systems biology is to join researchers from different disciplines into a single group. In line with this development, the IBIS team is a merger of an experimental biology group on the one hand, and a bioinformatics and biological modeling group on the other hand. In particular, the IBIS team is composed of members of the group of Hans Geiselmann at the Laboratoire Adaptation et Pathogénicité des Microorganismes of the Université Joseph Fourier (UJF, CNRS UMR 5163), and the network modeling and simulation group formerly part of the HELIX project-team at INRIA Grenoble - Rhône-Alpes, a group coordinated by Hidde de Jong. Both groups include researchers and technicians from other institutes, such as CNRS and the Université Pierre Mendès France (UPMF). The two groups have established a fruitful collaboration, which has resulted in more than 40 peer-reviewed publications in journals, conferences, and books since 2000.[1]

Hidde de Jong is the head of the IBIS team and Hans Geiselmann its co-director. The experimental biology component of IBIS also remains part of the Laboratoire Adaptation et Pathogénicité des Microorganismes, and Hans Geiselmann continues to represent this group in the interactions with the laboratory and university administration.

2.2. Highlights of the year

Version 7.0 of the modeling and simulation tool GENETIC NETWORK ANALYZER (GNA) was released in July 2009 and is described in paper for *BMC Bioinformatics* published this year. Version 3.0 of WELLREADER, a program for the analysis of reporter gene data, was released in November 2009 and is the subject of a recently accepted paper in *Bioinformatics*.

3. Scientific Foundations

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[1] See http://ibis.inrialpes.fr for a complete list.

*Figure 1. The ibis was an object of religious veneration in ancient Egypt, particularly associated with the god Thoth. Thoth was seen, among other things, as a god of the **measurement and regulation of events**. Here Thoth is shown in human form with the face of an ibis. (Sources: http://en.wikipedia.org/wiki/Ibis, http://en.wikipedia.org/wiki/Thoth, and http://www.shoarns.com).*

3.1. Models: Development and reduction of models of bacterial regulatory networks

Participants: Valentina Baldazzi, Sara Berthoumieux, Jérôme Izard, Johannes Geiselmann, Hidde de Jong, Yves Markowicz, Delphine Ropers [Correspondent].

The adaptation of bacteria to changes in their environment is controlled on the molecular level by large and complex interaction networks involving genes, mRNAs, proteins, and metabolites (Figure 2). The elucidation of the structure of these networks has much progressed as a result of decades of work in genetics, biochemistry, and molecular biology. Most of the time, however, it is not well understood how the response of a bacterium to a particular environmental stress emerges from the interactions between the molecular components of the network. This has called forth an increasing interest in the mathematical modeling of the dynamics of biological networks, in the context of a broader movement called systems biology.

In theory, it is possible to write down mathematical models of biochemical networks, and study these by means of classical analysis and simulation tools. In practice, this is not easy to achieve though, as quantitative data on kinetic parameters are usually absent for most systems of biological interest. Moreover, the models include a large number of variables, are strongly nonlinear and include different time-scales, which make them difficult to handle both mathematically and computationally. A possible approach to this problem has been to use approximate models that preserve essential dynamical properties of the networks. Different approaches have been proposed in the literature, such as the use of approximations of the typical response functions found in gene and metabolic regulation and the reduction of the model dimension by decomposing the system into fast and slow variables. These reductions and approximations result in simplified models that are easier to analyze mathematically and for which parameter values can be more reliably estimated from the available experimental data.

Model reduction approaches are exploited in IBIS to gain a better understanding of the ability of the *E. coli* to adapt to a various nutritional and other environmental stresses, such as carbon, phosophate, and nitrogen starvation. We are particularly interested in gaining a better understanding of the role of the so-called global regulators of gene expression in shaping the survival strategies of the bacteria. Moreover, we study the interactions between metabolism and gene expression in the adaptation of *E. coli* to changes in available
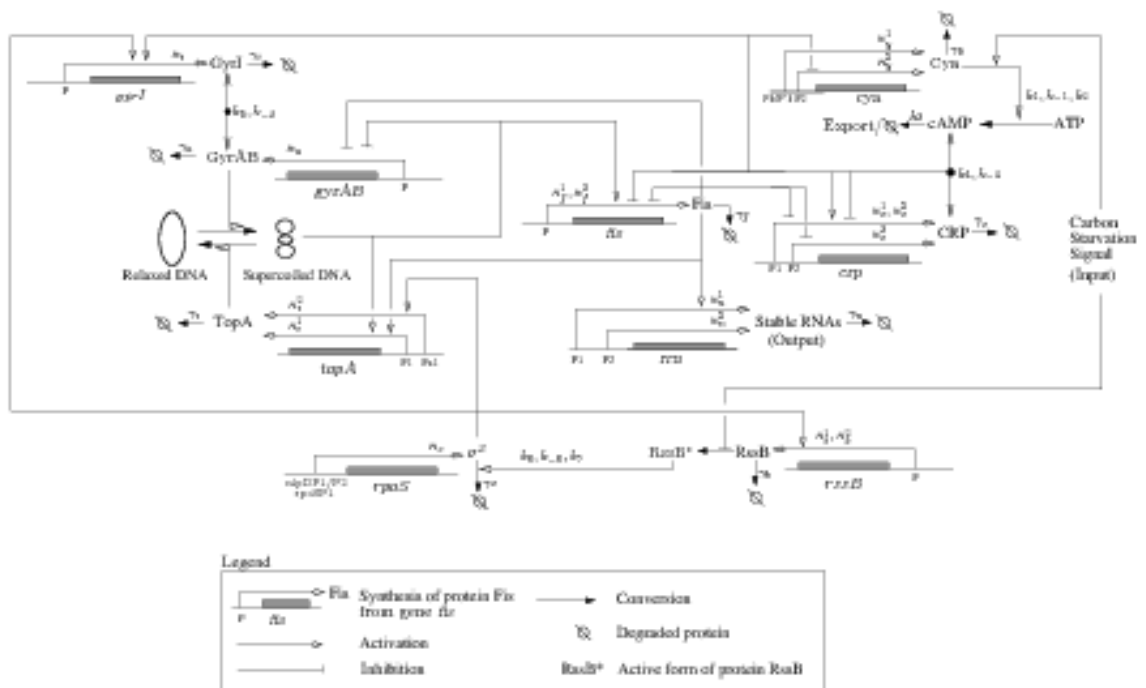
Figure 2. Network of key genes, proteins, and regulatory interactions involved in the nutritional stress network in E. coli (figure adapted from Monteiro et al., Bioinformatics, 24(16):i227-i233, 2008).

carbon sources. These topics are studied in collaboration with the HELIX and COMORE project-teams at INRIA.

3.2. Methods: Analysis, simulation, and identification of bacterial regulatory networks

Participants: Valentina Baldazzi, Sara Berthoumieux, Bruno Besson, Eugenio Cinquemani, Johannes Geiselmann, Hidde de Jong [Correspondent], Pedro Monteiro, Michel Page, François Rechenmann, Delphine Ropers, Woeh-Fu Wang.

Computer simulation is a powerful tool for explaining the capability of bacteria to adapt to sudden changes in their environment in terms of structural features of the underlying regulatory network, such as interlocked positive and negative feedback loops. Moreover, computer simulation allows the prediction of unexpected or otherwise interesting phenomena that call for experimental verification. The use of simplified models of the stress response networks makes simulation easier in two respects. In the first place, model reduction restricts the class of models to a form that is usually easier to treat mathematically, in particular when quantitative information on the model parameters is absent or unreliable. Second, in situations where quantitative precision is necessary, the estimation of parameter values from available experimental data is easier to achieve when using models with a reduced number of parameters.

Over the past few years, we have developed in collaboration with the COMORE project-team a qualitative simulation method adapted to a class of piecewise-linear (PL) differential equation models of genetic regulatory networks. The PL models, originally introduced by Leon Glass and Stuart Kauffman, provide a coarse-grained picture of the dynamics of genetic regulatory networks. They associate a protein or mRNA concentration variable to each of the genes in the network, and capture the switch-like character of gene regulation by means of step functions that change their value at a threshold concentration of the proteins. The advantage of using PL models is that the qualitative dynamics of the high-dimensional systems are relatively simple to analyze, using inequality constraints on the parameters rather than exact numerical values. The qualitative dynamics of genetic regulatory networks can be conveniently analyzed by means of discrete abstractions that transform the PL model into so-called state transition graphs.
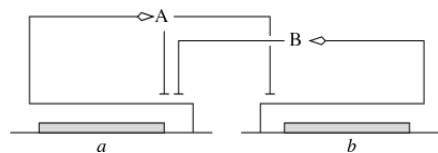
$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_a^2) \, s^-(x_b, \theta_b) - \gamma_a \, x_a$$

$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_a^1) - \gamma_b \, x_b$$

$$s^+(x, \theta) = \begin{cases} 1, & \text{if } x > \theta \\ 0, & \text{if } x < \theta \end{cases} \tag{2}$$

$$s^-(x, \theta) = 1 - s^+(x, \theta)$$

(a)   (b)

Figure 3. (a) Example of a genetic regulatory network of two genes (a and b), each coding for a regulatory protein (A and B). Protein B inhibits the expression of gene a, while protein A inhibits the expression of gene b and its own gene. (b) PLDE model corresponding to the network in (a). Protein A is synthesized at a rate κ_a, if and only if the concentration of protein A is below its threshold θ_a^2 ($x_a < \theta_a^2$) and the concentration of protein B below its threshold θ_b ($x_b < \theta_b$). The degradation of protein A occurs at a rate proportional to the concentration of the protein itself ($\gamma_a \, x_a$).

The development and analysis of PL models of genetic regulatory network has been implemented in the qualitative simulation tool GENETIC NETWORK ANALYZER (GNA) (Section 4.1). GNA has been used for the analysis of several bacterial regulatory networks, such as the initiation of sporulation in *B. subtilis*, quorum sensing in *P. aeruginosa*, the carbon starvation response in *E. coli*, and the onset of virulence in *E. chrysanthemi*. GNA is currently distributed by the Genostar company, but remains freely available for academic research. The analysis of models of actual bacterial regulatory networks by means of GNA leads to large state transition graphs, which makes manual verification of properties of interest practically infeasible. This has motivated the coupling of GNA to formal verification tools, in particular model checkers that allow properties formulated in temporal logic to be verified on state transition graphs. This is part of on-going collaborations with the POP-ART and VASY project-teams at INRIA Grenoble - Rhône-Alpes.

Recent advances in molecular biology and biophysics have led to new techniques for measuring cellular processes in real-time on the molecular level (Section 3.3). The data sources that are becoming available by means of these techniques contain a wealth of information for the quantification of the interactions in the regulatory networks in the cell. This has stimulated a broadening of the methodological framework in IBIS, extending the scope from qualitative to quantitative models, and from PL models to nonlinear ODE models. It has notably initiated work on what is the bottleneck in the practical use of these models, the structural and parametric identification of bacterial regulatory networks from time-series data, in collaboration colleagues from the University of Pavia (Italy) and ETH Zürich (Switzerland). This raises difficult problems related to identifiability, measurement noise, heterogeneity of data sources, and the design of informative experiments that are becoming increasingly prominent in the systems biology literature.

3.3. Data: High-precision measurements of gene expression in bacteria

Participants: Guillaume Baptist, Sara Berthoumieux, Johannes Geiselmann [Correspondent], Jérôme Izard, Hidde de Jong, Stephan Lacour, Yves Markowicz, Corinne Pinel, Caroline Ranquet-Brazzolotto, Delphine Ropers.

The goals of a model are to describe the functioning of bacterial regulatory networks in a way that helps to understand the underlying mechanisms and to predict the behavior of the system in new situations. In order to achieve these goals, we have to confront model predictions with experimental observations. This requires the availability of high-precision measurements of gene expression and other key processes in the cell.

We have resorted to the measurement of fluorescent and luminescent reporter genes, which allow monitoring the expression of a few dozens of regulators in parallel, with the precision and temporal resolution needed for the validation of our models. More specifically, we have constructed transcriptional and translational fusions of key regulatory genes of *E. coli* to fluorescent and luminescent reporter genes (Figure 4). The signals of these reporter genes are measured *in vivo* by an automated, thermostated microplate reader. This makes it possible to monitor in real time the variation in the expression of a few dozens of genes in response to an external perturbation. We have developed an experimental pipeline that resolves most technical difficulties in the generation of reproducible time-series measurements. The pipeline comes with data analysis software that converts the measurements into representations of the time-course of promoter activities that can be compared with model predictions (Section 4.3). In order to obtain rich information about the network dynamics, we have begun to measure the expression dynamics in both wild-type and mutant cells, using an existing *E. coli* mutant collection. Moreover, we have developed tools for the perturbation of the system, such as expression vectors for the controlled induction of particular genes.

While reporter gene systems allow the dynamics of gene expression to be measured with high precision and temporal resolution on the level of cell populations, they do not provide information on all variables of interest though. Additional technologies may complement those that we have developed in our laboratory, such as mass-spectrometry tools in proteomics and metabolomics that are able to quantify the amounts of proteins and metabolites, respectively, in the cells at a given time-point. In addition, for many purposes it is also important to be able to characterize gene expression on the level of single cells instead of cell populations. This requires experimental platforms that measure the expression of reporter genes in isolated cells by means of fluorescence and luminescence microscopy. IBIS has access to these technologies through collaborations with other groups
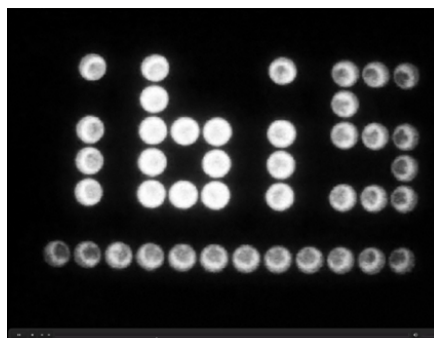
Figure 4. Playful illustration of the principle of reporter genes (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid containing the luciferase operon (luxCDABE) under control of the acs promoter. This promoter is positively regulated by the CRP-cAMP complex. When bacteria have metabolized all the glucose, the cAMP concentration increases quickly and activates the global regulator CRP which turns on the transcription of the luciferase operon producing the light. The glucose concentration increases from left to right on the microplate, so its consumption takes more time when going up the gradient and the letters appear one after the other. The luciferase protein needs reductive power (FMNH2) to produce light. At the end, when acetate has been depleted, there is no more carbon source in the wells. As a consequence, the reductive power falls and the "bacterial billboard" switches off. Source: Guillaume Baptist.

on the local and national level, such as the INSA de Toulouse and the Laboratoire de Spectrométrie Physique at the Université Joseph Fourier.

4. Software

4.1. Genetic Network Analyzer (GNA)

Participants: Bruno Besson, Hidde de Jong [Correspondent], Pedro Monteiro, Michel Page, François Rechenmann, Delphine Ropers.

GENETIC NETWORK ANALYZER (GNA) is the implementation of a method for the qualitative modeling and simulation of genetic regulatory networks developed in the IBIS project. The input of GNA consists of a model of the regulatory network in the form of a system of piecewise-linear differential equations, supplemented by inequality constraints on the parameters and initial conditions. From this information, GNA generates a state transition graph summarizing the qualitative dynamics of the system. GNA is currently distributed by the company Genostar, but remains freely available for academic research purposes. The current version is GNA 7.0. In comparison with the previously distributed versions, GNA 7.0 has the following additional functionalities. First, the specification of the qualitative dynamics of a network in temporal logic, using high-level query templates. Second, the automated verification of these properties on the state transition graph by means of standard model-checking tools, either locally installed or accessible through a remote web server. For more information, see http://www-helix.inrialpes.fr/gna.

4.2. ISee

Participant: François Rechenmann [Correspondent].

The aim of ISEE (IN SILICO BIOLOGY E-LEARNING ENVIRONMENT) is to explain the principles of the main bioinformatics algorithms through interactive graphical user interfaces and to illustrate the application of the algorithms to real genomic data. Written in Java, ISEE defines a generic framework for combining algorithms with courses. More precisely, the environment implements the metaphor of a lab notebook: the left pages present and explain the experiments to be carried out by the student, whereas the right pages display the progress of these experiments, *i.e.*, the execution of the associated algorithms. In its present state, the environment offers different algorithmic modules structured into three main chapters: sequence comparison, statistical analysis of DNA sequences for the identification of coding regions, and basic pattern-matching algorithms including the use of regular expressions. These and other algorithms have been integrated in two original practical courses. The first one is an introduction to the statistical analysis of genetic sequences and leads the student to the identification of the origin of replication within bacterial genomes. The second one shows the student how to identify coding regions in bacterial genomes and to characterize their products. The latter course was developed in collaboration with the CCSTI ("Centre de Culture Scientifique Technique et Industrielle") in Grenoble, which used ISEE for its "École de l'ADN". For more information, see: http://ibis.inrialpes.fr/article124.html.

4.3. WellReader

Participants: Guillaume Baptist, Bruno Besson, Frédéric Boyer, Johannes Geiselmann, Jérôme Izard, Hidde de Jong [Correspondent], Delphine Ropers.

WELLREADER is a program for the analysis of gene expression data obtained by means of fluorescent and luminescent reporter genes. WELLREADER reads data files in an XML format or in a format produced by microplate readers, and allows the user to detect outliers, perform background corrections and spline fits, compute promoter activities and protein concentrations, and compare expression profiles across different conditions. WELLREADER has been written in MATLAB and is available under an LGPL licence, both as source code (M files) and compiled code (platform-specific binary files). For more information, see: https://prabi1.inrialpes.fr/trac/wellreader/wiki.

5. New Results

5.1. Models: Development and reduction of models of bacterial regulatory networks

The new results in 2009 concern (i) the development or the extension of models of the *E. coli* carbon starvation response and their experimental validation and (ii) the reduction of previously-developed models to simplified nonlinear models.

5.1.1. *Development and validation of models of the carbon starvation response in E. coli*

In the bacterium *E. coli*, the adaptation to the carbon-source availability is controlled by a complex network involving signaling cascades, metabolic reactions, and gene expression (Section 3.1). The different modes of regulation are interwoven to such an extent that it is difficult to understand how they coordinate the response of *E. coli* cells to changes in nutrient conditions. To address this question we previously developed kinetic models that focus on the role played by global regulators in the adaptation to the carbon-source availability. The initial confrontation of model predictions with experimental data obtained in the laboratory of Hans Geiselmann was inconclusive and has brought to the fore phenomena that are interesting, but difficult to explain by means of our models. We have therefore focused on the development of new hypotheses and new models based on them.

In particular, the data suggest that the interactions between global regulators of transcription are not sufficient to account for the control of gene expression during the growth transitions of *E. coli*. The analysis of the patterns of expression of global regulators duringthe growth transitions of *E. coli* suggests an effect of the cellular gene expression machinery, which controls the global regulators at the transcriptional, translational, and stability levels. To verify this hypothesis, Cao Yan, Vaibhhav Sinha, and Delphine Ropers have been developing kinetic models of the global control of gene expression by the cellular machinery, using standard approaches in biochemistry. These models, taking the form of systems of nonlinear differential-algebraic equations, describe the rate of change of the concentrations of the network components. They allow to analyze the effect of the regulation of the concentration and activity of the RNA polymerase and the ribosomes in the global control of gene expression during growth recovery following a carbon upshift. These models are also used for the development of growth control strategies in the ColAge project (Section 7.1).

In parallel, we have continued the investigation of the role of metabolic and genetic interactions in the control of gene expression during the growth transitions of *E. coli*. Global regulators of transcription control the synthesis of enzymes as well as their own synthesis, while the products of the metabolism control the activity of transcription factors. In order to work with models that can be easily manipulated and calibrated with experimental data, Guillaume Baptist, Mohammed El Amine Youcef, and Delphine Ropers have developed simplified kinetic models. These ODE models focus on components which have been experimentally identified as key players in the adaptation to carbon-source availability. They are tailored to a specific biological question, such as the regulation of the expression of the gene *acs*, coding for an enzyme in acetate metabolism, under different growth conditions, *e.g.*, glucose exhaustion. These ODE models have proven useful in the explanation of experimental observations on *acs* expression and the generation of new hypothesis on the regulation of this gene. A paper on the latter study is in preparation.

5.1.2. *Reduction of models of the carbon starvation response*

We previously developed a model of the control of glycolysis and neoglucogenesis in *E. coli*, composed of 39 nonlinear differential equations with 159 parameters. Most of the parameters are unknown and the time constants of the system span several orders of magnitudes. In order to reduce the model to a more manageable form, Valentina Baldazzi and Hidde de Jong, in collaboration with other IBIS members and Daniel Kahn (BAMBOO), have used an approach based on the systematic derivation of direct and indirect interactions in a genetic regulatory network from the underlying biochemical reaction network. They have shown that model reduction based on quasi-steady-state approximations in combination with sensitivity criteria are able to uncover such interactions. A paper describing this method and its application to the analysis of the network controlling glycolysis and neoglucogenesis in *E. coli* has been submitted for publication. As an extension of this work, Valentina Baldazzi has developed in the context of the EC-MOAN project a PL model describing the adaptation of the expression levels of the genes encoding global regulators and enzymes following a shift from glycolytic to neoglucogenetic growth.

A basic module in all of the above models, are equations describing the process of gene expression, that is, the synthesis of a protein from a DNA template. Classically, gene expression is modeled as a single step or assumed to be composed of two steps, transcription and translation. Especially when studying high-dimensional models it may be worthwhile to lump the entire gene expression step into a single step. Chris Barot, Hidde de Jong, Jean-Luc Gouzé (COMORE), and Eric Benoît (Université de la Rochelle) have studied the conditions under which it is justified to make these simplifications, using the mathematical framework of singular perturbations, and verified if these conditions are satisfied in actual time-series data sets. An article on this study is in preparation.

5.2. Methods: Analysis, simulation, and identification of bacterial regulatory networks

Our efforts focused on several on-going projects around the development of methods and tools for the analysis of bacterial regulatory networks: (i) the development of the tool GNA into an integrated modeling and simulation environment, including network definition and formal verification modules, and (ii) the structural and parametric identification of the networks.

5.2.1. *Integrated modeling, simulation, analysis, and verification in GNA*

Within the framework of two European projects, COBIOS and EC-MOAN (Section 7.2), IBIS has continued to extend the GENETIC NETWORK ANALYZER (GNA) modeling and simulation tool. GNA uses PL models to qualitatively model the dynamics of genetic regulatory networks (Section 4.1).

In the EC-MOAN project, we have continued to make formal verification technology available to the users of GNA, in collaboration with Radu Mateescu of the VASY project-team. We have notably finished the extension of GNA with a formal verification module that allows the user to specify dynamic properties of genetic regulatory networks by means of so-called patterns, high-level query templates that capture recurring questions of biological interest. The patterns can be automatically translated to temporal logic, for instance the CTRL (Computation Tree Regular Logic) that Pedro Monteiro developed in the framework of his PhD thesis. The formal verification module allows the user of GNA to have access to formal verification tools through a service-oriented architecture. This architecture, which has been completely implemented by Estelle Dumas, Pedro Monteiro, and Michel Page, integrates modeling and simulation clients like GNA to model-checker servers, via an intermediate request manager. In particular, the client can perform formal verification requests through the web, which the request manager dispatches to an appropriate model-checker server. When the model-checker server has answered the request, the results are sent back to the client for display and further analysis in the graphical user interface of the tool. The service-oriented architecture is modular and general, and has the advantage of reusing existing formal verification technology as much as possible. In collaboration with Gregor Goessler (POP-ART) and Grégory Batt (CONTRAINTES), we have developed efficient, implicit encodings of the state transition graphs representing the qualitative network dynamics, so as to optimize the interactions between GNA and the model-checker servers. These implicit encoding are currently being exploited for the development of methods for the verification of incompletely specified PL models of genetic regulatory networks.

Version 7 of GNA which includes the connection with model-checking tools through the service-oriented architecture, has been deposited at the Agence pour la Protection des Programmes (APP) and released in the summer of 2009. A paper for *BMC Bioinformatics* on GNA 7, which illustrated its use for the qualitative analysis of the *E. coli* carbon starvation network (Section 5.1), was accepted this year [5]. In parallel, a paper on the use of the temporal logic CTRL for the formal verification of genetic regulatory logic is under revision for a special journal issue associated with the conference Computational Methods in Systems Biology, which was held in Rostock in 2008. We also finished a chapter on version 7 of GNA that will be published in a forthcoming book volume on the modeling of bacterial molecular networks [13].

In the COBIOS project, IBIS and Genostar jointly developed a conceptual model to represent bacterial regulatory networks. The model has been implemented into a library called IOGMANETWORK, using the underlying entity-relationship data and knowledge model of Genostar's IOGMA platform. This work has notably involved Bruno Besson, Hidde de Jong, Michel Page, François Rechenmann, and engineers of Genostar. In the period covered by this report, IBIS contributed to the test, debugging, and extension of this library, among other things assuring the compatibility with the Systems Biology Graphical Notation (SBGN) standard. A stable version of the library has been completed. Most of the work in COBIOS, however, has concerned the integration of the IogmaNetwork library as a network editor into GNA. The aim is to support the entire modeling process from the structural definition or design of networks to their simulation and analysis within a single environment. Moreover, the integration of the IogmaNetwork library allows the user of GNA to access other modules in the Iogma environment, such as PathwayExplorer. The integration has involved a complete reorganization of the architecture of GNA and the development of a new graphical user interface. This allows the modeler to flexibly move back and forth between the definition of a network, the reduction of this network to a form compatible with the piecewise-linear models supported by GNA, and the semi-automatic translation of the network structure into a model. A version of GNA including the network editor will be available by the end of the COBIOS project in 2010.

5.2.2. *Identification of bacterial regulatory networks*

The work on PL models and GNA has been inspired by the fact that in most cases only steady-state, discrete-type experimental data is available, providing a qualitative description of the system dynamics. Modern experimental techniques are increasingly providing high-quality, time-course observations of the network dynamics and are thus paving the way for the use of quantitative models (Section 3.1). A major problem is the identification of such quantitative models from the data, either estimating parameter values or inferring the structure of the network. In addition to using standard heuristic techniques for system identification, several novel methods for the identification of bacterial regulatory networks are under development within IBIS.

A first effort in this direction has been carried out in the context of the ANR project MetaGenoReg (Section 7.1), led by Daniel Kahn (BAMBOO). Matteo Brilli (BAMBOO) has developed an approximate model of central metabolism of *E. coli*, using so-called linlog functions to approximately describe the rates of the enzymatic reactions. We use metabolome and transcriptome data sets from MetaGenoReg partners and the literature to estimate the parameters of the linlog models, a task greatly simplified by the mathematical form of the latter. One of the problems encountered is the occurrence of missing data, due to experimental problems or instrumental failures. We try to address the problem of missing data using approaches from the statistical literature based on EM algorithms. The objective is to ultimately integrate the resulting model of metabolism, assumed to be at quasi-steady state, into a kinetic model describing the regulation of gene expression. The model reduction and parameter estimation challenges encountered in this context form the subject of the PhD thesis of Sara Berthoumieux.

While this approach is mostly concerned with parametric identification, a second effort addresses the combined structural and parametric identification of bacterial regulatory networks from times-series data, continuing ideas originally developed in the HYGEIA project. Eugenio Cinquemani, who joined IBIS in November 2009, has continued a collaboration with Giancarlo Ferrari-Trecate (University of Pavia, Italy) and Riccardo Porreca (ETH Zürich, Switzerland). We consider the problem of learning ODE models where regulatory interactions are captured by sums and products of sigmoidal nonlinearities. To this end, statistical regression and hypothesis testing tools for the identification of best fitting models of appropriate complexity are used. One major challenge is the intractable number of alternative model structures that should be compared on the basis of the data. We are developing methods for the *a priori* selection of the most relevant model structures based on the use of biological knowledge and on a data preprocessing step inspired by model verification. An article on this work is currently under submission.

5.3. Data: High-precision measurements of gene expression in bacteria

During this past year we spent much effort in improving the quality and extent of the experimental observations by (i) modifying the reporter gene system and validating key steps of the reporter system, (ii) constructing expression vectors that allow subtle perturbations of the regulatory system, and (iii) developing a new method for identifying the pertinent genes to be included in a model.

5.3.1. *Improvements of gene expression measurements*

We have improved the gene expression measurements by exploring new variants of the green fluorescent protein (GFP), by transferring some of the constructions to the chromosome and by measuring and validating intermediate steps of gene expression. This work involves Corinne Pinel and Caroline Ranquet-Brazzolotto, post-doctoral researcher in the framework of the EC-MOAN project.

We are using two types of reporter genes: bacterial luciferase and variants of the GFP. The luciferase is much more sensitive than GFP, but since it is an enzyme, its activity depends not only on gene expression, but also on the metabolic state of the cell. We therefore systematically construct an identical reporter vector using GFP. An important consideration for the measurements is the half-life of the reporter protein. Rapid changes in gene expression are difficult to measure with very stable reporter proteins. We have therefore engineered GFP by adding (or not) a degradation tag in order to control its half-life.

Our reporter constructs are located on a low copy-number plasmid (about 20 copies per cell). However, we can not formally exclude that some of our experimental manipulations change the plasmid copy number. In this case, we would misinterpret signal changes as changes in gene expression when really they only reflect changes in plasmid copy number. We have therefore constructed two "platforms" on the chromosome of *E. coli* into which we can transfer our reporter constructs, assuring that there is exactly one copy per cell. We have adapted this new system to the constraint, particularly important for the *gfp* constructs, that a single copy decreases the signal intensity by a factor of 20.

The relevant variable in our models, described by most equations, are the promoter activity of a gene under investigation and the concentration of its protein. However, reporter genes measure the accumulation of the reporter protein after transcription of the gene into mRNA and translation of this mRNA into protein. In order to relate the signal (reporter gene activity) to the relevant variables (promoter activity, protein concentration) we have to make assumptions about the kinetics of the intermediate steps. In order to validate these assumptions we have directly measured the relevant quantities (mRNA accumulation and stability) for one model gene.

An article describing the validation of the models used for the interpretation of the reporter gene data is currently under revision. The computer tool WELLREADER (Section 4.3) assists the user in transforming the primary data in biologically relevant variables. Version 3 of this tool has been deposited at the APP and was released in the fall of this year. An application note on WELLREADER has been accepted for publication [2]."

5.3.2. *System perturbations*

In order to probe the system properties, we have to perturb the system and observe the dynamics of the system response by means of reporter genes. Until now, most of our perturbations consisted in changing the nutrient source of the bacteria. This affects the activity of a small number of genes. In order to perturb the system more systematically, Caroline Ranquet-Brazzolotto and Corine Pinel have constructed a series of expression vectors that allow the controlled induction of any particular gene at a precise moment during the time course of the experiment. We have characterized the induction profile of our expression vector and have constructed a second vector, using a different external signal, which will allow us to externally control the expression of two different genes in the bacterium.

We have also modified some of the proteins that are expressed from these vectors by adding a degradation tag to the proteins. The second vector can then be used to express a specific protease that will destroy the target protein when the external signal is given. We are currently calibrating this system, developed by Guillaume Baptist in the framework of his PhD thesis. These constructs will allow us to perturb the system in many different and very controlled ways, in exact analogy to modifications that can be introduced into the dynamical model of the network.

Somewhat 'cruder' perturbations consist in eliminating an entire node of the network. This corresponds biologically to the deletion of the corresponding gene. We have explored many of these single mutants (see below), but we have also begun to construct multiple mutants, where two or more genes of the network are removed. This serves to further probe the system behavior and test model predictions. This serves to further probe the system behavior and test model predictions. An even more radical approach consists in the redesign of part of this network, as is for instance undertaken in the PhD thesis of Jérôme Izard, funded by INRIA in the framework of the Action d'Envergure ColAge (Section 7.1)."

5.3.3. *Detecting the input function of a gene*

Another important task for the modeling and the biological understanding of a process is to well delimit the system boundaries. While high-throughput methods for detecting the target of a particular regulator are now classical (typically, DNA microarrays are used to study the effect of a particular mutation on the expression of all genes of a genome), no efficient method exists to determine the regulators that affect, directly or indirectly, the expression of a gene under investigation (the 'input function').

We have developed such a technique by making use of the Keio mutant collection of *E. coli* and devising a method for efficiently transforming our reporter plasmid into more than 4000 different clones. We have optimized the different steps of the procedure: transformation, detection of different signals (luminescence or coloration), image analysis, mutant selection and dynamical measurements of gene expression in the selected mutants. We have applied the method for identifying regulators that control the expression of genes that are critical for growth on acetate by *E. coli*, and regulators that modulate the expression of genes responsible for extracellular structures, so-called curli. This work has involved all members of the experimental side of IBIS and is currently being prepared for publication.

In both cases we have confirmed known regulatory mechanisms, but we have also discovered novel inputs to the regulation of these genes. We have established collaborations with other laboratories in France and Europe, specialized in the measurement of metabolites or metabolic activities, that will complement our measurements of gene expression. This direction is paralleled on the modeling side by the inclusion of metabolic reactions in the regulatory scheme and the resulting development of coarse-grained models and methods for model reduction (Section 3.1).

6. Contracts and Grants with Industry

6.1. Genostar

Participant: François Rechenmann.

Genostar, an INRIA start-up created in 2004, is a company developing software and solutions for the management and analysis of genomic and post-genomic data. The software has been developed, from 1999 to 2004, by the Genostar consortium (INRIA, Institut Pasteur, and the two biotech companies Genome Express and Hybrigenics) and by the HELIX project-team. It includes several modules originally developed by HELIX , notably GenoAnnot, GenoLink, GenoBool and GenoExpertBacteria. The modules have been integrated in the Iogma bioinformatics environment, which also includes the modeling and simulation tool GNA developed by members of IBIS (Section 4.1). François Rechenmann is scientific consultant of the company and members of IBIS and Genostar together collaborate in the COBIOS project (7.2). For more information, see http://www.genostar.com.

7. Other Grants and Activities

7.1. National projects

Project name	MetaGenoReg – Towards an understanding of the interrelations between metabolic and gene regulation: E. coli carbon metabolism as a test case
Coordinator	D. Kahn
IBIS participants	V. Baldazzi, G. Baptist, S. Berthoumieux, E. Cinquemani, J. Geiselmann, H. de Jong, Y. Markowicz, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers, W.-F. Wang
Type	ANR BIOSYS (2006-2010)
Web page	Not available

Project name	ColAge – Lifespan control in bacteria: Natural and engineering solutions
Coordinator	H. Berry
IBIS participants	G. Baptist, E. Cinquemani, J. Geiselmann, H. de Jong, J. Izard, S. Lacour, C. Pinel, D. Ropers, W.-F. Wang
Type	Action d'Envergure INRIA-INSERM (2008-2012)
Web page	http://colage.saclay.inria.fr

Project name	Séminaire grenoblois des systèmes complexes
Coordinators	O. François, A. Girard et D. Ropers
IBIS participants	D. Ropers
Type	Funding by Institut des Systèmes Complexes de Lyon (IXXI)
Web page	http://www.ixxi.fr/Seminaires.php

7.2. European projects

Project name	EC-MOAN: Scalable modeling and analysis techniques to study emergent cell behavior: Understanding the E. coli stress response
Coordinator	J. van der Pol
IBIS participants	V. Baldazzi, G. Baptist, J. Geiselmann, H. de Jong, Y. Markowicz, P. Monteiro, M. Page, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers
Type	European Commission, FP6 NEST (2007-2010)
Web page	http://wwwhome.cs.utwente.nl/~ecmoan1/

Project name	COBIOS: Engineering and Control of Biological Systems: A New Way to Tackle Complex Diseases and Biotechnological Innovation
Coordinator	D. di Bernardo
IBIS participants	B. Besson, H. de Jong, M. Page, F. Rechenmann, D. Ropers
Type	European Commission, FP6 NEST (2007-2010)
Web page	http://www.cobios.net

8. Dissemination

8.1. Editorial, organizational, and reviewing activities

Hidde de Jong

Type	Journal, conference, agency
Member Editorial Board	Journal of Mathematical Biology
Member Editorial Board	ACM/IEEE Transactions on Computational Biology and Bioinformatics
Member Program Committee	AIME 09, GENSIPS 09, HiBi 09, JOBIM 09, QR 09
Member Evaluation Committee	Netherlands Organisation for Scientific Research (NWO), Computational Life Science
Member Recruitment Committee	Université Joseph Fourier, assistant-professor bioinformatics/mathematical biology
Coordinator (with S. Robin)	Working group on Transcriptome, protéome, modélisation, inférence et analyse des réseaux biologiques of GDR CNRS 3003 Bioinformatique moléculaire
Member PhD Committee	Jonathan Fromentin (Ecole Centrale de Nantes), Jan van Klinken (University of Siena, Italy), Aurélien Naldi (Université de la Méditerranée - Aix-Marseille II), Hayssam Soueidan (Université de Bordeaux I)
Member Organization Committee	Journée biologie synthétique et micro et nanotechnologies, OMNT, CEA Grenoble
Project reviews	ANR, Université Paris-Orsay)

Hans Geiselmann

Type	Journal, conference, agency
Member PhD Committee President Recruitment Committee Member Organization Committee	Letizia Tagliabue (Universita degli Studi di Milano, Italy) Université Joseph Fourier, assistant-professor cellular biology Journée biologie synthétique et micro et nanotechnologies, OMNT, CEA Grenoble
Project reviews	ANR, Dutch National Science Foundation (NWO), Fonds National Suisse

Delphine Ropers

Type	Journal, conference, agency
Member Organization Committee Member Program Committee Project reviews	SeMoVi (Séminaire de Modélisation du Vivant) Satellite meeting of JOBIM 2009, JOBIM 2010 ANR

8.2. Other administrative activities

Hans Geiselmann is leader of the Control of Gene Expression group in the "Laboratoire Adaptation et Pathogénie des Microorganismes" (UMR 5163).

Yves Markowicz is a national representative of the UNSA trade union.

Hidde de Jong is correspondent of the Department of International Relations of INRIA at the Grenoble - Rhône-Alpes research center. He also coordinated the INRIA evaluation seminar on the Bioinformatics and Computational Biology theme.

François Rechenmann is leader of the editorial committee of the Interstices website (http://interstices.info). He is also president of the committee awarding CORDI-S PhD grants at INRIA Grenoble - Rhône-Alpes.

Delphine Ropers represents INRIA Grenoble - Rhône-Alpes in the scientific board of IXXI, the Complex Systems Institute in Lyon (http://www.ixxi.fr).

8.3. Seminars and PhD thesis defenses

Valentina Baldazzi

Title	Event and location	Date
The carbon assimilation network in *E. coli* is densely connected and largely sign-determined"	ECCS09, Warwick University, England	Sep. 2009
The carbon assimilation network in *E. coli* is densely connected and largely sign-determined"	XXIX Séminaire Société Francophone de Biologie Théorique, Saint Flour	Jun. 2009
Mechanical Statistics of Unzipping: Bayesian Inference of DNA Sequence	MAPMO, Université d'Orleans	Jun. 2009

Guillaume Baptist

Title	Event and location	Date
" Biologie synthétique "	Seminar Laboratoire Adaptation et Pathogénicité des Microorganismes (LAPM), Grenoble	Apr. 2009

Hidde de Jong

Title	Event and location	Date
Qualitative modeling and simulation of bacterial regulatory networks	Invited talk during 2nd Workshop on Computational Models for Cell Processes, Eindhoven (the Netherlands)	Oct. 2009
Qualitative modeling and simulation of bacterial regulatory networks	Seminar Ecole Polytechnique Fédérale de Lausanne (Switzerland)	May 2009
Qualitative modeling and simulation of bacterial regulatory networks	Seminar Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg (Germany)	July 2009
Hybrid Models of Genetic Regulatory Networks: Theory and Applications	Ecole Centrale de Nantes	March 2009

Hans Geiselmann

Title	Event and location	Date
Experimental analysis and modeling of the central genetic regulatory network of *Escherichia coli*	National Taiwan University, Taipei, Taiwan	May 2009
The RpoS-Crl regulon of *Escherichia coli*	University of Milano, Milano, Italy	Sep. 2009

Stéphan Lacour

Title	Event and location	Date
Mutations affecting congo-Red binding in *Escherichia coli*	Eurobiofilms 2009, Rome (Italy)	Sep. 2009

Caroline Ranquet

Title	Event and location	Date
Studying regulatory networks in in *Escherichia coli*: genetics, metabolism, bioinformatics	NIH Seminar, Bethesda, USA	Sep. 2009

François Rechenmann

Title	Event and location	Date
Lanalyse de linformation génétique la bioinformatique	Lycée Champollion, Grenoble	Jan. 2009
Informatique et sciences du vivant	Formation des professeurs de l'Académie de Versailles, Rocquencourt	Jun. 2009
Algorithmes et génomes	Séminaire Inter-académique de mathématiques, Lycée International, Europôle, Grenoble	Nov. 2009

Delphine Ropers

Title	Event and location	Date
Qualitative simulation of the carbon starvation response in *Escherichia coli*	seminar INRA, Toulouse	Feb. 2009
Kinetic modeling of biochemical systems in bacteria	15th IFAC Symposium on Systems Identification (SYSID 2009), Saint Malo	Jul. 2009
A l'interface de disciplines (avec G. Beslon, LIRIS, Lyon)	Une Heure Ensemble, INRIA Grenoble - Rhône-Alpes, Montbonnot	Dec. 2009

8.4. Popular science writing

The members of IBIS are actively involved in the dissemination of research results in systems biology and bioinformatics to a wider, non-specialist audience.

François Rechenmann is a regular contributor to *La Recherche* [14] and coordinated in 2009 a special issue of *DocSciences* entitled "Le numérique et les sciences du vivant" (http://www.docsciences.fr/). Delphine Ropers and Hidde de Jong made contributions to the special issue [15]. François Rechenmann is also involved in the development of an In Silico biology e-learning environment (ISee), which explains the principles of the main bioinformatics algorithms and illustrates their use on real data (Section 4.2). He is leader of the editorial committee of the Interstices website (http://interstices.info). Interstices offers pedagogic presentations of research themes and activities in the computer science domain.

8.5. Teaching

Four members of the IBIS team are either full professor, associate professor or assistant professors at the Université Joseph Fourier or the Université Pierre Mendès-France in Grenoble. They therefore have a full teaching service (at least 192 hours per year) and administrative duties related to the organization and evaluation of the university course programs on all levels (from BSc to PhD). Besides the full-time academic staff in IBIS, the following people have contributed to courses last year.

Guillaume Baptist

Subject	Year	Location	Hours
Prokaryotic genetics	1-4	Department of Biology, Université Joseph Fourier	73

Sara Berthoumieux

Subject	Year	Location	Hours
Parametric curves and differential equations	2	Université Joseph Fourier	32

Hidde de Jong

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	INSA de Lyon	16
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	12

Pedro Monteiro

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	4

Delphine Ropers

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	7
Modeling and simulation of genetic regulatory networks	4	Université Joseph Fourier	5

Hidde de Jong developed with Daniel Kahn a module on the modeling of genetic and metabolic networks at INSA de Lyon.

9. Bibliography

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[2] F. BOYER, B. BESSON, G. BAPTIST, J. IZARD, C. PINEL, D. ROPERS, J. GEISELMANN, H. DE JONG. *A MATLAB program for the analysis of fluorescence and luminescence reporter gene data*, in "Bioinformatics", 2010, In press.

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International Peer-Reviewed Conference/Proceedings

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[9] R. PORRECA, S. DRULHE, H. DE JONG, G. FERRARI-TRECATE. *Inferring structure and parameters of genetic regulatory networks: an approach based on hybrid systems*, in "Workshop on Classification and Forecasting Models, ECOBIOSYS 2009, Milano, Italy", 2009 IT .

[10] D. ROPERS, V. BALDAZZI, H. DE JONG. *Reduction of a kinetic model of the carbon starvation response in Escherichia coli*, in "Proceedings of the 15th IFAC Symposium on System Identification, SYSID 2009, Saint Malo, France", 2009.

National Peer-Reviewed Conference/Proceedings

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yeast cells: qualitative modeling and simulation, in "Proceedings of the Congresso Nacional MicroBiotec'09, Vilamoura, Portugal", 2009 PT .

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