From Qualitative to Quantitative Models of Gene Regulatory Networks in Bacteria (Extended Abstract)

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The adaptation of bacteria to changes in their environment involves adjustments in the expression of genes coding for enzymes, regulators, membrane transporters, *etc*. [14], [26], [17]. These adjustments are controlled by gene regulatory networks ensuring the coordinated expression of clusters of functionally related genes. A realistic view of gene regulatory networks does not only include direct interactions resulting from transcription regulation, but also indirect regulatory interactions mediated by metabolic effectors and signaling molecules. The network controlling carbon uptake in the bacterium *Escherichia coli* is a case in point. Global regulators like Crp control expression of enzymes in carbon metabolism, while intermediates of the latter pathways control the expression of global regulators. For instance, the phosphorylation of EIIA activates adenylate cyclase (Cya) to produce cAMP which is required for the activation of Crp. Ignoring indirect interactions during the analysis of the network dynamics may lead crucial feedback loops to be missed.

I will describe how indirect interactions between genes can be derived from a model of the underlying biochemical reaction network by combining quasi-steady-state approximations expressing weak assumptions on time-scale hierarchies in the system [23], [22], [27] with sensitivity criteria from metabolic control analysis [22], [24]. When applied to a model of the carbon assimilation network in *E. coli*, the derived gene regulatory network is shown to be densely connected, contrary to what is usually assumed. Moreover, the network is largely sign-determined, meaning that the signs of the indirect interactions are fixed by the flux directions of biochemical reactions, independently of specific parameter values and rate laws. An inversion of the fluxes following a change in growth conditions may affect the signs of the indirect interactions though. This leads to a feedback structure that is at the same time robust to changes in the kinetic properties of enzymes and that has the flexibility to accommodate radical changes in the environment [2].

In theory, it is possible to write down mathematical models of the gene regulatory networks, and study their dynamical properties by means of classical systems analysis tools. In practice, this is not easy to achieve though, as quantitative data on kinetic parameters are usually absent, the models have a large number of variables, and they are strongly nonlinear. This has motivated the interest in qualitative models which, from incomplete knowledge of the system, are able to provide a coarse-grained picture of its dynamics (*e.g.*, [11], [18], [32]). A variety of qualitative models for gene regulatory networks have been proposed in the literature, but in my presentation I will focus on piecewise-linear (PL) differential equation models introduced by Leon Glass and Stuart Kauffman [19].

The defining characteristic of PL models is that they capture direct or indirect regulatory effects by means of step functions that change their value in a switch-like manner at threshold concentrations of the regulators. The step functions are approximations of the sigmoidal response functions often occurring in gene regulation [29]. The use of step functions allows the qualitative dynamics of the PL models to be analyzed, even in higher-dimensional systems (*e.g.*, [4], [5], [12], [13], [15], [19], [21], [20], [28]). The thresholds of the concentration variables define a hyperrectangular partition of the state space, such that in every region not located on a threshold, the PL model reduces to an analytically solvable system of differential equations. Moreover, in every such region the derivatives (trends) of the concentration variables have a determinate sign, which is shown to be invariant for rather weak constraints on the parameter values. The definition of these constraints can generally be inferred from available data in the experimental literature or by intuitive reasoning, even in the absence of quantitative information on parameter values. The properties of the PL models motivate a discrete representation of the continuous dynamics, described by a state transition graph. The states in the graph correspond to the regions in the state space, while the transitions between the states arise from solutions of the PL model that enter one region from another. Each transition thus corresponds to a discrete event, namely the crossing of a threshold by one or more concentration variables, possibly entailing a change in the derivative of these variables.

Genetic Network Analyzer (GNA) is a computer tool specifically developed for the analysis of gene regulatory networks by means of PL models [8], [9], [25]. It allows the user to define a regulatory network, build a model of this network, determine the steady states of the system, generate a state transition graph starting from an initial state (qualitative simulation of the network dynamics), and analyze the latter graph using model-checking tools. GNA has been used for the analysis of a number of bacterial regulatory networks, among other things the carbon starvation response in *Escherichia coli* [31]. I show how qualitative modeling and simulation of the network of global regulators has allowed us to identify essential features of the transition between exponential and stationary phase of the bacteria and to make

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new predictions on the gene expression dynamics following the exhaustion of glucose.

In order to test these predictions, we have monitored in real-time, using fluorescent and luminescent reporter plasmids in combination with an automated microplate reader, the expression level of the major global regulators (Crp, Fis, RpoS, GyrA, TopA, ...) involved in the carbon starvation response. Fluorescent and luminescent reporter genes are popular tools for quantifying gene expression. The underlying principle of the technology is to fuse the promoter region and possibly (part of) the coding region of a gene of interest to a reporter gene. The expression of the reporter gene generates a visible signal (fluorescence or luminescence) that is easy to capture and reflects the expression of the gene of interest. Mathematical models are critical for inferring biologically relevant quantities, such as (relative) protein concentrations and promoter activities, from the primary data (see [10], [16], [34] and references therein). I will illustrate the methodological principles and compare the gene expression profiles thus obtained with those predicted from the qualitative model of the *E. coli* network.

The new technologies developed in molecular biology and biophysics for measuring cellular processes at the molecular level in real time provide a wealth of information for the quantification of the interactions in the regulatory networks governing the adaptation of bacterial physiology to environmental changes. They make it increasingly possible to ”assign numbers to the arrows” in network diagrams [30] and move from qualitative to quantitative models. I will describe some issues that currently constitute methodological bottlenecks for the structural and parametric identification of bacterial regulatory networks from experimental data (see [1], [3], [6], [7], [33] for reviews).

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