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Attractor computation using interconnected Boolean networks: Testing growth rate models in *E. Coli*

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ABSTRACT

Boolean networks provide a useful tool to address questions on the structure of large biochemical interactions since they do not require kinetic details and, in addition, a wide range of computational tools and algorithms is available to exactly compute and study the dynamical properties of these models. A recently developed method has shown that the attractors, or asymptotic behavior, of an asynchronous Boolean network can be computed at a much lower cost if the network is written as an interconnection of two smaller modules. We have applied this methodology to study the interconnection of two Boolean models to explore bacterial growth and its interactions with the cellular gene expression machinery, with a focus on growth dynamics as a function of ribosomes, RNA polymerase and other "bulk" proteins inside the cell. The discrete framework permits easier testing of different combinations of biochemical interactions, leading to hypotheses elimination and model discrimination, and thus providing useful insights for the construction of a more detailed dynamical growth model.

1. Introduction

Large networks with complex interactions are hard to analyze in detail, but logical and discrete models can facilitate this task. Based essentially on the structure and topology of the network interactions, logical models provide qualitative information on the dynamical properties of the system [1,2], which can be used for model discrimination and guidance in model improvement. There are many recent examples of applications of discrete models including *Drosophila* embryo pattern formation [3,4], yeast cell cycle [5], T-cell response [6], or an apoptosis network [7].

Boolean networks are a class of logical models whose variables are described in terms of only two levels (1 or 0; presence or absence; "on" or "off"), which have been useful for biochemical systems [8]. The dynamics of a Boolean model is determined by specifying an updating mode, most commonly synchronous (all nodes updated simultaneously) or asynchronous (only one node updated at any given instant). Since the state space is finite, the dynamics can be represented in terms of a transition graph, which can be studied using some classical algorithms from graph theory [9]. Other, more specific tools are available for an exact and rigorous analysis of the transition graph [10], computation of attractors (or asymptotic behavior) [11], and other properties [12]. In addition, a wide range of computer tools are available for simulation and analysis of discrete models [13], model reduction [14], or model checking [15].

It is clear that discrete models are not appropriate to finely describe the behavior of a system, since continuous effects are difficult to reproduce with such models (such as whether an oscillation is sustained or damped), but they are useful to

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verify whether a given network of interactions is feasible and compatible with known properties of the system. This is a first step towards the construction of a more detailed and informed model.

As an application, we will analyze a network of interactions involved in determining bacterial growth of *Escherichia Coli*, which varies nonlinearly with different factors, such as availability of nutrients or the concentration of the necessary enzymes and proteins needed for cell division [16,17]. Mathematical models have been developed to describe and reproduce several regulatory modules and their response to nutrient availability [18,19]. One of the least understood aspects in these studies remains the actual modeling of bacterial growth: while it is clear that growth depends on the general availability of “bulk” proteins, ribosomes, and RNA polymerase, it is difficult to find a reasonable mathematical model that reproduces all these effects [20]. In many cases, growth is considered to be a given constant and the model is designed to reproduce a single phase of bacterial growth.

Here, we propose to test and study a dynamical function for bacterial growth in terms of the major components involved in bacterial cell division, that is, gene transcription (RNA polymerase) and translation (ribosomes). To test the feasibility of mathematical growth functions, we will focus on a qualitative model of the network involved in the carbon starvation response [18] and its interconnection with a basic model describing the dynamics of ribosomes and RNA polymerase (see Section 3).

We will use two methods for analysis of qualitative systems (see Section 2): first, a method that transforms piecewise affine (PWA) systems into discrete and then Boolean models [21,22]; and, second, a recently developed method to compute the attractors of an interconnection of two Boolean modules [11,23]. Our analysis generates a general view of the dynamical properties of a model which is a first step towards verifying the feasibility of the model’s structure – by comparing to experimental observations – and facilitates hypotheses testing. The results indicate that at least two (positive) qualitative levels for growth rate (such as “high” and “intermediate” rates) are needed in order to reproduce both the stationary and exponential growth phases (see Section 4).

2. Methodology

In this section, we briefly recall two mathematical methods which are very useful for the analysis of qualitative systems and, in particular, interconnections of Boolean models.

2.1. From discrete to Boolean models

Although Boolean variables can only take the values 0 or 1, it is nevertheless possible to construct Boolean models that describe variables with a discrete number of values [24,21]. Consider a discrete model $\Sigma_{disc} = (\Omega_d, F_d)$, with variables $V = (V_1, \dots, V_n)'$, state space $\Omega_d = \prod_{i=1}^{n}\{0, 1, 2, \dots, d_i\}$, where $d_i \in \mathbb{N}$ is the number of levels of variable V_i $(i = 1, \dots, n)$, and a state transition table $F_d : \Omega_d \to \Omega_d$. The state of the system at the next instant $k + 1$ is given as a function of the state of the system at the current instant k, according to the rules F_d, using the notation:

$$V^+ = \tilde{F}_d(V).$$

Throughout this paper, the function $\tilde{F}_d$ is obtained from F_d by assuming an *asynchronous dynamic updating rule*, that is, exactly one variable is updated at any given time:

$$V^+ \in \left\{ W \in \Omega_d : \exists k \text{ s.t. } W_k = (F_d)_k(V) \neq V_k \text{ and } W_j = V_j,\ \forall j \neq k \right\}. \tag{1}$$

Furthermore, for a more realistic model, we consider that each variable V_i can only switch from its current level to an immediately adjacent level [12], that is:

$$V_i^+ \in \{V_i - 1, V_i, V_i + 1\}, \quad \forall i. \tag{2}$$

The idea is to create an extended Boolean model $\Sigma_{bool} = (\Omega_b, F_b)$ where each discrete variable V_i is represented by d_i Boolean variables, for instance, $\{X_{i,1}, \dots, X_{i,d_i}\}$, so that the state space of the model is $\Omega_b = \{0, 1\}^{d_1 + \dots + d_n}$. There are several possible ways to convert the discrete into the Boolean variables, but here we chose to use the same criterion as in [21] which stipulates that

$$V_i = k \quad \Leftrightarrow \quad (X_{i,1} = \dots = X_{i,k} = 1,\ X_{i,k+1} = \dots = X_{i,d_i} = 0), \tag{3}$$

meaning that a variable i is at a state k if and only if all the first k Boolean variables are ON. In particular, note that this criterion implies the partition of the state space of the extended Boolean model into *permissible and forbidden regions*:

$$\Omega_p = \{X \in \Omega_b : k < l \Leftrightarrow X_{i,k} \geq X_{i,l}\}, \qquad \Omega_f = \{0, 1\}^{d_1 + \dots + d_n} \setminus \Omega_p.$$

Thus, to generate the Boolean transition table F_b we need to guarantee that no transitions from a permissible to a forbidden state take place. The method described in [21] deals with this problem in a natural way, and guarantees that no transitions from permissible to forbidden states take place.