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Reliability of regulatory networks and its evolution

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ABSTRACT

The problem of reliability of the dynamics in biological regulatory networks is studied in the framework of a generalized Boolean network model with continuous timing and noise. Using well-known artificial genetic networks such as the repressilator, we discuss concepts of reliability of rhythmic attractors. In a simple evolution process we investigate how overall network structure affects the reliability of the dynamics. In the course of the evolution, networks are selected for reliable dynamics. We find that most networks can be easily evolved towards reliable functioning while preserving the original function.

1. Introduction

Biological systems are composed of molecular components and the interactions between these components are of an intrinsically stochastic nature. At the same time, living cells perform their tasks reliably, which leads to the question how reliability of a regulatory system can be ensured despite the omnipresent molecular fluctuations in its biochemical interactions.

Previously, this question has been investigated mainly on the single gene or molecule species level. In particular, different mechanisms of noise attenuation and control have been explored, such as the relation of gene activity changes, transcription and translation efficiency or gene redundancy (Ozbudak et al., 2002; Raser and O'Shea, 2005; McAdams and Arkin, 1999). Apart from these mechanisms acting on the level of the individual biochemical reactions, also features of the circuitry of the reaction networks can be identified which aid robust functioning (Barkai and Leibler, 1997; Alon et al., 1999; von Dassow et al., 2000). A prime example of such a qualitative feature that leads to an increased stability of a gene's expression level despite fluctuations of the reactants is negative autoregulation (Becskei and Serrano, 2000). At higher levels of organization, the specific linking pattern of the larger biochemical regulatory networks can further contribute to the overall robustness. In comparative computational studies of several different organisms, it has been shown that among those topologies that produce the desired functional behavior only a small number also displays high robustness against parameter variations. Indeed, the experimentally observed networks rank high among these robust topologies (Kollmann et al., 2005; Wagner, 2005a; Ma et al., 2006).

However, most current models are based on the deterministic dynamics of differential equations. Modeling of the intrinsic noise associated with the various processes in the network requires an inherently stochastic modeling framework, such as stochastic differential equations or a master equation approach (Thattai and van Oudenaarden, 2001; Kepler and Elston, 2001; Ozbudak et al., 2002; Rao et al., 2002). These complex modeling schemes need a large number of parameters such as binding constants and reaction rates and can only be conducted for well-known systems or simple engineered circuits. For generic investigations of such systems, coarse-grained modeling schemes have been devised that focus on network features instead of the specifics of the reactions involved (Bornholdt, 2005).

To incorporate the effects of molecular fluctuations into discrete models, a commonly used approach is to allow random flips of the node states. Several biological networks have been investigated in this framework and a robust functioning of the core topologies has been identified (Albert and Othmer, 2003; Li et al., 2004; Davidich and Bornholdt, 2008). However, for biological systems, the perturbation by node state flips appears to be an unrealistic type of noise: in real organisms, concentrations and timings fluctuate, while the qualitative state of a gene is often quite stable. A more realistic form of fluctuations than macroscopic (state flip) noise should allow for microscopic fluctuations. This can be implemented in terms of fluctuating timing of switching events (Klemm and Bornholdt, 2005b; Chaves et al., 2005; Braunewell and Bornholdt, 2007). The principle idea is to allow for fluctuations of event times and test whether the

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dynamical behavior of a given network stays time ordered despite these fluctuations.

In this work we want to focus on the reliability criterion that has been used to show the robustness of the yeast cell-cycle dynamics against timing perturbations (Braunewell and Bornholdt, 2007) and investigate the interplay of topological structure and dynamical robustness. Using small genetic circuits we explore the concept of reliability and discuss design principles of reliable networks.

However, biological networks have not been engineered with these principles in mind, but instead have emerged from evolutionary procedures. We want to investigate whether an evolutionary procedure can account for reliability of network dynamics. A number of studies has focused on the question of evolution towards robustness (Wagner, 1996; Bornholdt and Sneppen, 2000; Ciliberti et al., 2007; Szejka and Drossel, 2007; Aldana et al., 2007). However, the evolution of reliability against timing fluctuations has not been investigated. First indications that network architecture can be evolved to display reliable dynamics despite fluctuating transmission times has been obtained in a first study in Braunewell and Bornholdt (2008). Using a deterministic criterion for reliable functioning, introduced in Klemm and Bornholdt (2005a), it was found that small networks can be rapidly evolved towards fully reliable attractor landscapes. Also, if a given (unreliable) attractor is chosen as the "correct" system behavior, it was shown that with a high probability a simple network evolution is able to find a network that reproduces this attractor reliably, i.e. in the presence of noise.

Here, we use a more biologically plausible definition of timing noise to investigate whether a network evolution procedure can generate robust networks. We focus on the question whether a predefined network behavior can be implemented in a reliable way, just utilizing mutations of the network structure. We use a simple dynamical rule to obtain the genes' activity states, such that the dynamical behavior of the system is completely determined by the wiring of the network.

2. Model description

2.1. Boolean dynamics

A standard approach to computer simulations of molecular biological systems starts from chemical master equations and their explicit stochastic modeling, e.g. via Monte Carlo algorithms (Gillespie, 1977). However, such methods need a large number of parameters and detailed knowledge about the system in order to completely describe the system dynamics. As an alternative, for gaining first, qualitative insights into the dynamics of genetic regulatory systems it has proven useful to apply strongly coarse-grained models (Bornholdt, 2005).

Boolean networks, first introduced by Kauffman (1969) as anecdotal models of gene regulation based on random networks, have emerged as a successful tool for qualitative dynamical modeling and have been successfully employed in models of regulatory circuits in various organisms such as *Drosophila melanogaster* (Albert and Othmer, 2003), *Saccharomyces cerevisiae* (Li et al., 2004), *Arabidopsis thaliana* (Espinosa-Soto et al., 2004), and *Schizosaccharomyces pombe* (Davidich and Bornholdt, 2008). In this class of dynamical models, genes, proteins, and mRNA are modeled as discrete switches which assume one of only two possible states. Here, the active state represents a gene being transcribed or molecular concentrations (of mRNA or proteins) above a certain threshold level. Thus, at this level, a regulatory network is modeled as a simple network of switches.

Time is modeled in discrete steps and the state of all nodes is updated at the same time depending only on the state of all nodes at the previous time step according to the wiring of the network and the given Boolean function at each node.

When such a system is initialized with some given set of node states, it will in general follow a series of state changes until it reaches a configuration that has been visited before (finite number of states). Because of the deterministic nature of the dynamics, the system has then entered a limit cycle and repeats the same sequence of states indefinitely (or keeps the same state, then called a fixed point attractor).

2.2. Stochastic dynamics

In the original Boolean model there are two assumptions that are clearly non-biological and are thus often criticized: (1) The synchronized iteration of the Boolean network in discrete time steps implies total synchrony of all components. (2) The binary (ON/OFF) node states which prohibit intermediate levels and gradual effects.

There have been various attempts at loosening these assumptions while keeping the simplicity of the Boolean models. It is a clear advantage of Boolean models that they operate on a finite state space. The synchronous timing, however, does not hold a similar advantage apart from computational simplicity. Models that overcome this synchronous updating scheme have been suggested in a variety of forms. In Chaves et al. (2006) different asynchronous schemes are used in the model of the fruit fly. The simplest asynchronous model keeps the discrete notion of time but lets events happen sequentially instead of simultaneously. A continuous-time generalization of Boolean models that is inspired by differential equation models has been suggested in Klemm and Bornholdt (2005b). Here, the discreteness of the node states is kept but the dynamics take place in a continuous time. In Klemm and Bornholdt (2005a) and Braunewell and Bornholdt (2008) the limit of infinitesimally small disturbances from synchronous behavior is investigated.

This concept of allowing variations from the synchronous behavior will also be used in this work. The principle idea is to use a continuous time description and identify the state of the nodes at certain times with the discrete time steps of the synchronous description (Glass, 1975). Further, an internal continuous variable is introduced for every node and the binary value of the node is obtained from this continuous variable using a threshold function. Now a differential equation can be formulated for the continuous variable.

This is pictured in Fig. 1. Here the internal dynamics and the resulting activity state of a node with just one input are shown for a given input pattern. The activator A of the node B is switched on (through a signal from another node, for example) at time $t = 1$ and stays on until it is switched off at time $t = 2$. In the Boolean description we would say node A assumes state $S_A = 1$ at time step 1 and at time step 2 switches to state $S_A = 0$. Node B would react by switching to state $S_B = 1$ at step 2 and to $S_B = 0$ at step 3. In the continuous version, we implement this by a delay time and a "charging" behavior of the concentration value of node B, driven by the input variable S_A. As soon as c_B crosses the threshold of $\frac{1}{2}$, the activity state of B switches to $S_B = 1$.

Let us formulate the time evolution of a system of such model genes by the set of delay differential equations as

$$\tau \frac{dc_i(t)}{dt} = f_i(t, t_d) - c_i(t). \quad (1)$$

Here, $f_i(t, t_d)$ denotes the transmission function of node i and describes the effect of all inputs of node i at the current time. The parameter τ sets the time scale of the production or decay process.