Relaxation dynamics and frequency response of a noisy cell signaling network

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We investigate the dynamics of cell signaling using an experimentally based Boolean model of the human fibroblast signal transduction network. We determine via systematic numerical simulations the relaxation dynamics of the network in response to a constant set of inputs, both in the absence and in the presence of environmental fluctuations. We then study the network’s response to periodically modulated signals, uncovering different types of behaviors for different pairs of driven input and output nodes. The phenomena observed include low-pass, high-pass, and band-pass filtering of the input modulations, among other nontrivial responses, at frequencies around the relaxation frequency of the network. The results reveal that the dynamic response to the external modulation of biologically realistic signaling networks is versatile and robust to noise.


One of the characteristic features of living cells is their ability to continuously monitor their environment and respond appropriately to extracellular signals, which instruct the cells to take decisions such as proliferating, stopping growth, secreting chemicals, or even committing suicide. This behavior is mediated by signal transduction networks, which are composed of large numbers of interacting proteins. These networks have an input layer consisting of receptor proteins on the cell membrane that activate upon binding extracellular signals and an output layer of enzymes and/or transcription factors whose activation produces physiological changes in the cell. Until recently, the structure of these networks was largely unknown, and as a result, their theoretical study had to assume random connectivity between the nodes (proteins). Nowadays, the rise of high-throughput screening techniques allows the mapping of signaling networks with unprecedented precision.

I. INTRODUCTION

Cellular behavior is regulated by intricate networks of genes and proteins that interact with each other in a usually nonlinear manner, subject to non-negligible amounts of random fluctuations, or noise, arising from the intrinsic stochasticity of cellular components. Furthermore, these regulatory networks are commonly embedded in a temporally variable environment, as a result, for instance, of circadian and ultradian rhythms affecting the organism to which the cell belongs. The question of how regulatory networks, and in particular signaling networks, respond to this dynamic driving is still open mostly due to an incomplete knowledge of the underlying complex pattern of interactions.

One type of simplified description of regulatory networks that aims at maintaining only the essential ingredients of the biochemical interactions is the Boolean networks (BNs). They were introduced by Kauffman1 as simple models to support the idea that life does not require precisely programed units to generate complex and diverse behaviors, but rather random juxtaposition of simple units could achieve the same result. In this type of model, the network nodes are genes or proteins that are either fully active or inactive. The activity of the whole system results from the dynamical interaction of the nodes dictated by a set of logic rules. The advantage of this type of model is that it is simple and does not require as many parameters as kinetic-based models.

Most of the studies dealing with BNs to date have considered random network topologies.2 Properties such as criticality,3 robustness,4–9 and scalability10,11 have been studied in this type of random BNs (RBNs). However, real gene regulatory or signal transduction networks can be very different from uniformly connected networks with random interactions. Furthermore, RBNs are autonomous, and in the absence of random effects, they evolve deterministically to a well-defined attractor from some specific initial conditions. This feature, which may hold for certain gene regulatory networks, is not fulfilled by signal transduction networks, which are subject to external signals that frequently vary in...
time. In fact, the main goal of signaling networks is to process the information carried by the input signals, which is usually dynamical, although this latter aspect has not been much considered so far. In this direction, a recent experimental work has studied the response of the NF-κB pathway to periodic pulsatile stimulation by TNF-α, revealing a non-trivial frequency response to the input period. The question then arises as to what determines this response to the signal frequency.

Recently, experimentally based BNs have proven to be a good paradigm for modeling certain biological processes. In fact, with the recent progress in the experimental characterization of biological processes, BN models have been successfully applied to the study of gene regulatory networks, cellular differentiation, evolution, and signal transduction in the cell. Those studies provided evidence that sequences of events can indeed be reproduced by simple discrete dynamic models such as BNs. In this work, we are interested in the dynamical behavior of a biologically realistic BN model of the signal transduction machinery in human fibroblasts. We focus on the relaxation dynamics of the signaling network, the robustness of this relaxation to noise, and its influence on the frequency response of the network to a periodic modulation of the input signals. This paper is organized as follows. In Sec. II, we describe the implementation of the BN and its intrinsic dynamics. In Sec. III, we present the results obtained when the model is stimulated with fixed external inputs and varying unstructured and structured inputs. Finally, we discuss the biological consequences of the results obtained in Sec. IV.

II. THE BOOLEAN NETWORK MODEL

Boolean networks are discrete dynamical systems composed of individual units, called nodes, connected with each other by directed links. In a BN, each of the network nodes is assumed to be either active (1) or inactive (0) and to evolve in a discrete-time basis according to a set of node-specific logic rules. Specifically, in a BN with \( N \) nodes, \( i = 1, \ldots, N \), the state of each node \( x_i \) evolves according to its corresponding logic rule, \( f_i \), which depends on the current states of its \( k_i \) incident nodes, \( x_1^{k_i}, \ldots, x_{k_i}^{k_i} \),

\[
x_i(t+1) = f_i(x_1^{k_i}(t), \ldots, x_{k_i}^{k_i}(t)).
\]

In the classical approach to the dynamics of BNs, all of the states of the network are updated in parallel at every time iteration, resulting in the dynamical evolution of the activity of the nodes. Thus, time evolves in a discrete manner, with no quantitative meaning being associated with it. Hence, the model represents dynamical behaviors in terms of sequences of biochemical events rather than actual time so that all references to time and frequency in what follows are given in terms of model iterations. For a given initial condition of the network, the states of all nodes evolve in a deterministic manner according to the iteration map defined by the logic rules.

Here, we focus on a BN model recently proposed by Helikar et al. that describes the signal transduction network of a typical human fibroblast. The model was built from manual inspection of a large body of literature, resulting in the network shown in Fig. 1, which contains nine inputs that feed signals to 130 internal nodes, linked with each other through a web of 542 interactions. The nature of these inputs is diverse and includes generic and specific stress signals (interleukin-1 and tumor necrosis factor, or IL-1/TNF), a growth factor (epidermal growth factor (EGF)), an ion channel (calcium pump), extracellular matrix components (ECM), and the four families of ligands that use G-protein coupled receptors. These families are known as the \( \alpha_x \), \( \alpha_y \), \( \alpha_z \), and \( \alpha_{12/13} \) ligand families and contain several neurotransmitters, cytokines, and hormones. Since the pattern of interactions within each of these families is conserved, the BN model contains a generic ligand/receptor pair for each of them. Some of the 130 internal network nodes are related to a well-defined cellular response, namely, programed cell death (Akt), gene transcription (Erk), cytoskeletal regulation (Rac, Cdc42), and cellular stress (SAPK, p38). Thus, these six proteins are considered to be output nodes of the network even though they have outgoing edges to other intermediate nodes of the network. Helikar et al. validated their model by comparing the predicted input/output behaviors of different pairs of nodes with the existing experimental data, and the network was seen to agree with biological results.

This BN model, based on empirical evidence, diverges in many aspects from the classical and well understood RBN models. First, as mentioned above, this model is nonautonomous since it receives multiple dynamic inputs. Second, its connectivity distributions (in and out degree distributions) are far from uniform. For instance, while there are 21 nodes with a single input, other nodes have up to 13 different inputs and 28 output nodes. Two features of the network make the analysis of its information processing capabilities challenging: (i) the network inputs can reach the output nodes in a relatively small number of steps and (ii) there is a large num-

FIG. 1. (Color online) The network model used in this work consists of 130 internal nodes and 9 input nodes, which represent protein species being either active (1) or inactive (0), connected through 542 interactions. Input signals (top) and output nodes (bottom) are labeled with abbreviations that are defined in the text.
ber of feedback and feedforward loops present, resulting from the high and nonuniform connectivity of this network.

In the original study, the signaling network shown in Fig. 1 was examined in order to describe the characteristic outputs reached under different input conditions. The study found that the network had a tendency to produce a relatively small number of highly occurring output activities. Here, we are interested on the dynamical aspects of the network.

A consequence of the fact that in autonomous BNs the state update rules are deterministic is that the dynamics of the node states is also deterministic. The only indeterminacy in the evolution of autonomous BNs is set by the initial conditions. In the case of nonautonomous (or forced) BNs, the model acts as a processing element that filters the information passing through it using its own dynamics. When the input states are fixed, the evolution of the network necessarily reaches a stationary attractor, constant or periodic, after a certain transient. We will now study the relaxation toward these attractors in the presence of two different types of stationary inputs (fixed or stochastic) and use that information to understand the response of the network to time-varying (periodic) inputs.

III. RESULTS

A. Constant inputs

The dynamical system introduced in Sec. II evolves in time. The nodes in the network change their activity according to the logic rules acting upon them, their current state, and the states of the input signals. Any combination of constant input signals transforms the system into a distinct autonomous system. A typical evolution for a combination of constant inputs is shown in Fig. 2(a). In this example, the system relaxes for a while, during a transient period, toward a stationary configuration in which some nodes are inactive (black in the figure), some are constantly active (white), and a few periodically alternate between active and inactive states. In the RBN literature, this is known as the ordered regime. These types of dynamics, with periodic attractors, are an immediate consequence of having a finite number of possible states, $2^N$, and deterministic evolution rules.

Since we are interested in the dynamics of this system, we start by considering the distribution of transient lengths obtained for fixed inputs. To calculate the transients, we search first the stable or periodic orbits reached by the system at long times. Once we identify the attractor for a given specific simulation condition, we define the transient length as the number of iterations needed to reach the attractor. This network accepts $2^N$ different combinations of input signals (the nine inputs are fixed to 0 or 1), which will necessary produce periodic or fixed orbits defining the corresponding relaxation (transitory) duration. A full exploration of the phase space of this system is computationally unfeasible. Hence, we partially explore this space by sampling 3000 realizations of combination of constant input signals. This represents a total number of $1.536 \times 10^6$ realizations of the dynamics. From these realizations, we have identified 264 206 different attractors. This result shows that the structure of the phase space is far from trivial. Furthermore, there is a large variation in the number of attractors found from each combination of input signals. For some input combinations, there are only 10 different attractors in the 3000 realizations, while for others the number of attractors reaches 2168. As for the transient lengths, Fig. 2(b) shows the histogram of transient durations measured from this set of simulations. The distribution exhibits both a sharp peak of transient lengths centered at around 10 iteration time steps (average and median transient lengths are 22.8 and 16, respectively) and a fat tail with approximately 25% of the simulations having transients larger than 25 iterations. Each individual input condition leads qualitatively to the same kind of distribution when only its initial conditions are varied.

Thus, interestingly, a network as complex as the one represented in Fig. 1 produces, in general, a very well-defined temporal sequence of responses to static input conditions. To some extent, this behavior underlies the reliability and adaptability of the system to changes in the external inputs: a well-defined response guarantees that the system will adapt its dynamics always at the same pace. A dispersion of several orders of magnitude in the response duration would produce a variety of evolutions for different conditions, which would make the interaction with other cellular processes difficult. On the other hand, we speculate that a
well-defined response allows for a predictable and simple behavior that may be easily coupled with other cellular mechanisms.

We also note that the distribution of periods for the limit cycle attractors is not as sharp as that of the transient lengths but shows certain dispersion, with several forbidden periodic orbit lengths (results not shown). However, here we do not concentrate in the network’s attractors because they are not robust in the presence of noise, as we discuss below. We will see in Sec. III B that the relaxation length, on the other hand, does persist in the presence of fluctuations.

Each realization of the dynamics of the network model can be considered as the dynamics of a single cell. To study the response of a population of cells, we average the activity of each node, \( i \), at every iteration, for the \( R=3000 \) realizations of the dynamics described above,

\[
X_i(t) = \frac{1}{R} \sum_{r=1}^{R} x_i^r(t).
\]  

The average activity, \( X_i(t) \), takes a value between 0 and 1, which quantifies the probability of the corresponding node to be active at time \( t \). The question now is whether the average activity behaves in the same manner as the single-cell evolutions that we have examined above and, in particular, if it exhibits a well-defined transient duration. It should be noted here that we are not coupling networks, we simply deal with statistics of the network over different initial conditions for fixed sets of inputs. Figure 3(a) shows the temporal evolution of the average activity of the six output nodes of the network, as defined in Fig. 1, for the same input conditions shown in Fig. 2. In this particular case, some of the output nodes exhibit a zero activity, while some others reach an approximately constant nonzero activity. Certain input combinations lead to oscillatory average activity levels in some nodes. So, also at the population level, the network shows that the average activity of nodes is highly dependent on the input combination of states. Nevertheless, the distribution of transient lengths at the population level (which we can measure as the maximum time required for every node to reach its final value), shown in Fig. 3(b), is similar to the distribution for single cells [cf. Fig. 2(b)].

B. Inputs with constant chatter level

The environment in which cells are embedded changes irregularly in time. As a result, the cell receives noisy, randomly fluctuating signals, which introduce new and usually fast time scales, affecting its dynamics. We now explore the consequences of introducing noisy inputs into the system. To that end, we add a certain amount of variability, which we term chatter, to the constant input states considered above. This is accomplished by setting a probability, \( q \), for an input to be active at a given iteration. We then produce a series of events using a Bernoulli distribution with \( q \) mean. The sequence of zeros and ones obtained in this way has an average equal to \( q \) and is used as the succession of states for each of the input nodes. The sequences are noisy in general, with the highest variability appearing for \( q=0.5 \), where the active and inactive states have the same probability, while the variability disappears for \( q=0 \) and \( q=1 \), where the activities are again fixed at a constant state. We consider in this article the case of maximum variability, \( q=0.5 \), for all input nodes.

In order to investigate the effect of noise on the response of the system, we have performed several simulations with different initial conditions and realizations of the chatter. An example of the evolution obtained for a given realization is given in Fig. 4(a). This plot shows that the chatter destroys the deterministic attractors obtained for constant inputs, as described in Sec. III B. At the population level, however, each node reaches an approximately constant average level of activity, as shown in Fig. 4(b), which plots the population activity of the six output nodes of the network (the intermediate nodes have a qualitatively similar behavior). A comparison between Figs. 3(a) and 4(b) indicates that the transients do not change qualitatively in the presence of chatter. As in the case of constant inputs, we can measure the population relaxation duration as the number of iterations needed for every node to reach its stationary average level. For the particular case in Fig. 4(b), the estimated transient length is 11, which falls within the distribution of deterministic transients shown in Fig. 3(b). As mentioned above, this robustness of the relaxation of the network dynamics in the presence of noise is consistent with the findings of Sec. III B.

![FIG. 3.](image)

FIG. 3. (Color online) Population dynamics of the system for constant inputs. (a) Population average for output nodes obtained from 3000 realizations of the network dynamics with different initial conditions and the same fixed combination of input states as in Fig. 2(a). Different combinations of fixed inputs lead to different population averages of the outputs. (b) Histogram of the transient lengths of the population dynamics for the 512 possible combinations of input states. Transients for the population dynamics are computed as the maximum transient lengths of the average activities of single nodes. These node-specific transients were calculated by first establishing the range of values of the stationary state (within a certain tolerance) and then finding the iteration at which the node’s average activity value enters this range and never leaves it again. The population relaxation durations are close to the median transient lengths of individual realizations of the network dynamics [cf. Fig. 2(b)]. As in Fig. 2(b), the inset in plot (b) depicts the histogram in doubly logarithmic scale. The plot indicates that this histogram has again a fat tail, although in this case the number of data points is too small to reveal a clear-cut power law.
The strength of the response at the input frequency, however, depends on the value of that frequency, indicating that the network does not respond equally well to all frequencies. To show that, we plot in Fig. 6 the values of the power spectral density of the average activity of all six output nodes at the input frequency \( v_0 \) when this frequency varies. The figure shows two example cases corresponding to the periodic modulation of two different input nodes and reveals a variety of frequency responses including low-pass, high-pass, and band-pass filtering, together with other more complex behaviors. In all cases, however, the frequency cutoffs are located in the range of \( v_0 = 0.05 - 0.20 \), which correspond precisely to periods in the range of 5–20 that are very close to the peak of the relaxation durations that we have observed in the previous sections.

It is interesting to note here the influence of chatter on the network’s response to external signals. As we have seen,
the noisy signal introduced by chatter destroys the structure of the deterministic attractors of the system. On the other hand, it maintains the characteristic relaxation times of the network and helps transducing structured inputs, in the form of periodic signals, into their corresponding outputs. To some extent, the dynamics in the periodically driven case becomes a sequence of relaxations between the two states involved in the driving signal. The synergistic effect between noisy and structured inputs found here is an example of integration of signals by cells. Given that cells operate in a noisy environment while being required to respond to temporally structured inputs, we can expect the results obtained here to be relevant in realistic settings.

IV. DISCUSSION

Signaling is a key cellular process. Only with a complex and efficient flow of information across their biochemical machinery are cells able to react to changing environments and to fulfill the basic operations needed to maintain life. This highly complex behavior cannot be built under the basis of isolated specialized processes, but must rely on a certain level of global coherence. Even though many studies in the past have been devoted to examine specific mechanisms in the signaling system of the cell, little attention has been paid until the past decade to the integration of the different parts of the signaling machinery. Here, we are interested in this global aspect of the signaling mechanisms. In order to handle the complexity of the signaling machinery, we consider a reduced version of the system by decreasing the number of details while still capturing the main properties of the process. Boolean networks provide us with a mathematical framework that, even though being extremely simple and very schematic, keeps the essence of the information processing underlying signaling activity. Within that context, we have used a recently developed Boolean network that models the signaling network in human fibroblast cells, and that has been compiled from an extensive experimental literature analysis. We have used this network to study the global response of the system to external inputs of various nature. Each type of input considered introduces information into the system at different scales. We have computed the response of a single cell, understood in our model as a single realization of the dynamics, and of cell populations, understood as the average of several realizations sharing equivalent input conditions. Even though it would be interesting to consider the coupling between the cells that form the population by different types of mechanisms (e.g., chemical diffusion of ligands), we have restricted the present study to situations in which there is no coupling among cells, which receive input signals only through their input nodes.

It is well known that random Boolean networks evolve, in the absence of noise, toward steady or periodic attractors. We observed this type of behavior in our (nonrandom) network model when the input states of the network are fixed. The network evolves from different initial conditions (which may correspond to different cellular states) toward specific attractors by relaxing its dynamics during a transient period. We computed the duration of these transients and observed that their distribution peaks at around 10–20 iteration units. Thus, this signaling network reacts to different kinds of fixed inputs within the same time scale. This characteristic time of the dynamical response of the network is relatively constant, which means that the network goes from any region in phase space to any other in a narrow range of iterations. This behavior is a manifestation of the responsiveness and reliability of the network. When considered as a population by averaging the evolution of all simulation realizations, the system behaves similarly.

Purely constant inputs are nevertheless not very realistic biologically. Cells work in an environment that changes in time due to biochemical noise, variations of control conditions, or even coupling with other cells in multicellular organisms. We have addressed this situation by including a signal with fast variability, the chatter, to the network inputs. We implement this random chatter through a probability to have an active state in every input node of the network. Noise introduces a fast time scale into the system that is transduced down the network and which might interfere with its information processing abilities, specially taking into account its high connectivity. This does not occur, however. The only effect of the input chatter is the elimination of the deterministic attractors of the network, while the transient lengths are kept essentially the same as in the case of fixed inputs.

In this scenario, where the presence of noise does not destroy the system’s responsiveness, we ask how the network processes a well-defined external signal at different frequencies. Our results show that at the population level, the network is able to process input signals with frequencies in the range of the network’s relaxation frequency. This fact has clear biological implications, as it establishes that a particular biological system, such a transduction network, has a limited dynamic range for which it is able to process input signals. A recent experimental study has revealed that the NF-κB pathway responds in a nontrivial manner to the frequency of periodic stimulation by TNF-α. Even though the components of that pathway are not included in our network (the input signal is) and the pathway considered is relatively small and intrinsically dynamical, the modeling results presented here could provide a framework for understanding how the frequency response of signaling networks is related to their relaxation properties. Also, this phenomenon partially explains why the network reaches a stationary state when only noise is present in the inputs since we observe that high frequencies are always filtered out. Furthermore, in some cases, noise is required for the network to transduce the oscillating input at the allowed range of frequencies.

The range of nontrivial frequency responses reported here (low-pass, band-pass, and high-pass) implies that the signaling network being considered does not behave simply as a linear cascade, since in that case, the information would be simply propagated with a certain delay, of the order of the cascade length. The average shortest path length from inputs to outputs in the network of Fig. 1 is 3.87, which is much shorter than the median transient lengths reported here. Thus, the shortest path lengths alone cannot account for the relaxation. One could also argue that because the initial conditions are random, the relaxation duration is governed by the maxi-
relaxation behavior that is robust to the presence of noise that integrate multiple dynamic inputs exhibit a well-defined future work. This type of question would be worth being studied in a

Note that the dynamic logic rules depend only on the states of its upstream nodes in the previous iteration time. However, especially if the dynamics of specific proteins are complex, certain inputs to the logic gates might be affected by delays: states of certain nodes may depend on states of other nodes several iterations time steps before. This type of relation could change the dynamics and could alter the time scales involved in the processing performed by the network. Such a behavior might be captured by our model if we consider the latter as a phenomenological version of the real dynamics, describing effective coupling relations between nodes. A very systematic approach to the problem we have attacked here would try to produce a model that takes into account these lags. However, measuring such interactions is currently very difficult, and it may not improve significantly the predictive power of a simplified view of reality such as the one adopted by the present Boolean network model. Noise, for instance, would probably counterbalance the influence of the causal relations introduced by delays. This type of question would be worth being studied in a future work.

In conclusion, our results show that signaling networks that integrate multiple dynamic inputs exhibit a well-defined relaxation behavior that is robust to the presence of noise (in the form of background chatter), while being flexible enough to provide the cell with a rich toolbox of frequency responses to periodic input signals. Given that the network studied here is based on the available experimental data, and that noisy and periodic oscillations are ubiquitous in cellular environments, we expect the knowledge gained from these results to be relevant to understanding the realistic behavior of cells.

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