

From topology to dynamics in biochemical networks

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Abstract formulations of the regulation of gene expression as random Boolean switching networks have been studied extensively over the past three decades. These models have been developed to make statistical predictions of the types of dynamics observed in biological networks based on network topology and interaction bias, p . For values of mean connectivity chosen to correspond to real biological networks, these models predict disordered dynamics. However, chaotic dynamics seems to be absent from the functioning of a normal cell. While these models use a fixed number of inputs for each element in the network, recent experimental evidence suggests that several biological networks have distributions in connectivity. We therefore study randomly constructed Boolean networks with distributions in the number of inputs, K , to each element. We study three distributions: delta function, Poisson, and power law (scale free). We analytically show that the critical value of the interaction bias parameter, p , above which steady state behavior is observed, is independent of the distribution in the limit of the number of elements $N \rightarrow \infty$. We also study these networks numerically. Using three different measures (types of attractors, fraction of elements that are active, and length of period), we show that finite, scale-free networks are more ordered than either the Poisson or delta function networks below the critical point. Thus the topology of scale-free biochemical networks, characterized by a wide distribution in the number of inputs per element, may provide a source of order in living cells. © 2001 American Institute of Physics. [DOI: 10.1063/1.1414882]

Abstract models of gene regulation networks suggest that real cells should display chaotic dynamics. Experimental evidence suggests otherwise. We propose that a distributed connectivity, shown to have profound effects in other complex networks, may be the source of order in the biochemical circuits that control cellular behavior.

I. INTRODUCTION

The human genome has now been sequenced and many other genome sequencing projects are nearing completion. Concurrently, methods to identify protein-protein interactions,¹ as well as trans-acting regulatory proteins and cis-acting regulatory binding sites on a genome-wide scale are being developed.^{2,3} Thus, the complete topology of gene expression networks and signal transduction pathways that control cellular behavior is beginning to materialize. What is the significance of this topology to the functioning of a cell? Cellular function depends on the dynamics of the mRNA and protein concentrations that comprise the biochemical networks that make up a cell. Thus, understanding the relationship between the topology of these biochemical networks and their dynamics may provide some insight into the regulatory organization found in biochemical networks.

The relationship between topology and the dynamical states of large biological networks has been studied using

abstract randomly constructed Boolean networks.⁴ These networks are characterized by a fixed number of inputs, K , per element and an interaction bias p .⁴ While this formulation has obvious limitations compared to models with more realistic representations of the chemical kinetics, a number of results have been shown to be robust in the transition to the more realistic piecewise linear and nonlinear equation formulation.⁵⁻⁷ While Bagley and Glass showed that some attractors in the Boolean switching network models are artifacts of the synchronous updating and discretization of state space and time, the classification of the dynamical attractors of particular networks was shown to remain invariant in the piecewise linear and nonlinear equations.⁶ Also, it is the only framework in which large-scale statistical properties can be studied readily. It was shown by Kauffman⁴ that increasing the number of inputs per element pushed the system through a transition from steady state dynamics to periodic and finally to “disordered” dynamics in which the length of the cycle grows exponentially with the number of elements in the system. For an equal distribution of “on” and “off” states for the activity of an element ($p=0.5$), the transition is predicted to occur at two inputs per element.

Recent experimental work suggests that the number of inputs per element in real biochemical networks is greater than two, leading to a prediction of “disorder” or chaotic dynamics in cells. However, this prediction does not agree with experimental observations. The biological processes

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studied thus far can be classified into the following dynamical states: those displaying fixed point behavior such as the lac Operon in *E. coli*,⁸ and those with periodic dynamics such as the mammalian cell cycle, circadian rhythms in *Drosophila*, the Glycolysis pathway, and Ca^{2+} signaling.⁹ Chaotic dynamics seems to be absent from the fundamental dynamical states of a normal cell.

The dynamics of cellular systems may depend on the connectivity of the underlying biochemical circuits. Recent research has begun to elucidate the topology of real cellular networks. This effort has led to the discovery of several examples of cellular networks that have a mean number of inputs larger than the critical value of two and that are characterized by wide distributions in connectivity. As evidence of distributed connectivity in real biochemical circuits, we cite three examples: metabolic networks,¹⁰ yeast protein–protein interaction networks,¹ and mammalian cell cycle networks.¹¹

Jeong *et al.*¹⁰ did an extensive study of metabolic networks in 43 different organisms. They found that all of these networks had power law distributions, with an average exponent of 2.2. A specific example is the *S. cerevisiae* metabolic network. This network has 561 elements, with a power law exponent of 2.0, corresponding to a mean of 4.20 inputs per element.

Using yeast 2-hybrid methods on a genome wide scale, researchers at Curagen and University of Washington have examined protein–protein interactions in yeast.¹ Using yeast 2-hybrid technology to detect *in vivo* protein–protein interactions, they estimated the mean connectivity for yeast to be between 1.8 and 3.3. Also, they found many examples of proteins that have far more interactions than the mean. Their data suggest that the yeast protein–protein network likely has a broad distribution of connectivity.

Finally, Kohn¹¹ has compiled a comprehensive map of known interactions in the mammalian cell cycle. Although the network and its topology are not completely known, the Kohn map can provide a useful estimate of the distribution of connectivity in the mammalian cell cycle, which is known to have kicked-periodic dynamics under normal conditions of growth factor and hormone controlled cell division. To establish the connectivity of this network, we counted the number of inputs and outputs per protein and gene. Figure 1(a) shows the histogram acquired from the Kohn map. The total number of elements is 100, with a mean connectivity of 3.65. Figure 1(b) illustrates the fitted power law of the distribution. The exponent is 1.12.

In the framework of Kauffman networks, the mean connectivities of these examples suggest that real biological networks should display “disordered” dynamics. The fact that biological networks display ordered dynamics forces the question of the origin of this order. Some have approached this question from the point of view of biases in interactions such as canalizing functions,¹² and internal homogeneity.¹² While this may account for the order of these systems, topology may also play a role. In particular, the assumption that biochemical networks have a fixed number of inputs has no biophysical basis and appears to be in contradiction to emerging experimental evidence.^{1,10,11} We expect that a dis-

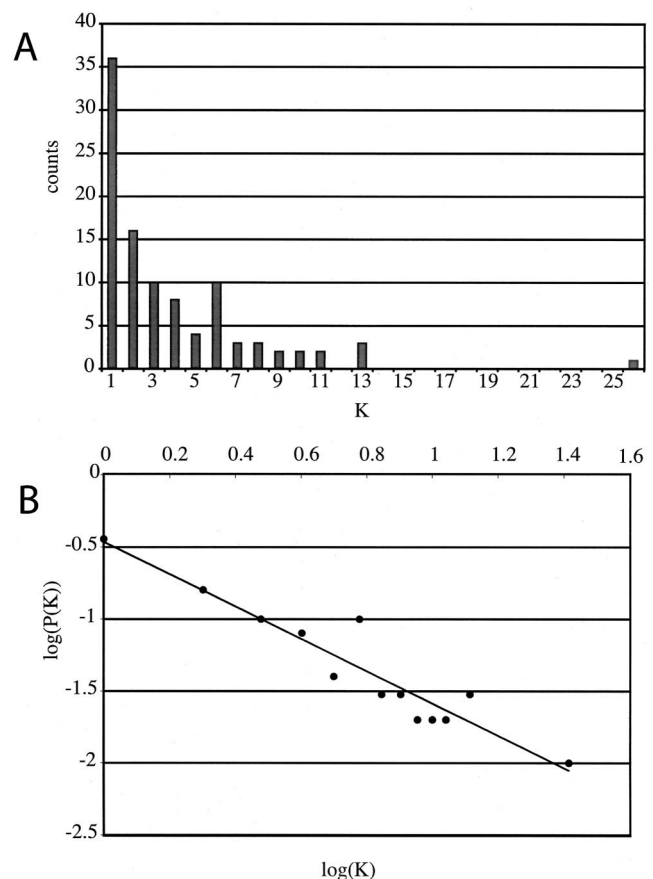


FIG. 1. (a) The histogram for the mammalian cell cycle obtained from the Kohn map (Ref. 12). Inputs to an element were defined as other elements that modified the behavior of the first element. Outputs were elements whose behavior was modified by the first element. We counted a total of 100 elements with at least one input in the Kohn map. (b) The dots are the data plotted in log–log format. The line is the fitted power law distribution for the network. The exponent is 1.12.

tribution in the number of inputs K may affect the dynamics of biochemical networks and may be a source of the order observed in real cells.

Recently, the effects of topology on properties of very large networks have been studied in the context of complex systems. Watts and Strogatz¹³ investigated “small world” networks, showing that seemingly small changes in topology in locally connected networks can greatly affect global properties such as average distance between nodes in a network and the rate of information propagation. Scale-free networks, characterized by power law distribution in connectivity, have also been studied. Several examples of large networks that show scale-free organization have been found: the World Wide Web, social networks, and power grid nets,¹⁴ as well as the previously mentioned metabolic networks. Such networks are robust and error tolerant.¹⁵ Also, a mechanism has been suggested whereby such organization could naturally arise in a growing network.¹⁴ However, the effect that network topology may have on dynamics has not been addressed.

Motivated by experimental evidence just highlighted, we investigate how a distribution of the number of inputs per element affects the dynamics of biochemical networks. We

investigate delta function, Poisson and power law distributions in the framework of Boolean networks. We analytically show that the critical value of the interaction bias parameter, p , is independent of the distribution in the limit of the number of elements $N \rightarrow \infty$. We also study these networks numerically using three different measures: types of attractors observed, fraction of active elements, and length of periodic orbits. We show that for finite networks below the critical point, a power law distribution produces networks that are more ordered than either the Poisson or delta function distributions. These results suggest that the recently characterized broad distribution in the number of inputs per element may provide a source of order in biochemical networks.

II. DEFINITION OF MODEL

The model that we study is a modification of the extensively studied Kauffman network.^{4,16–22} In this model, we allow each element of the network σ_i to take on the values of 0 or 1. The i th element receives input from K_i other elements in the network. These K_i inputs are chosen at random from the N elements. Self-inputs are allowed, but multiple inputs from the same element are not allowed. In our study, we choose K_i from a distribution $P(K)$, as opposed to the fixed K (delta function distribution) used in the original Kauffman model. $P(K)$ is nonzero only for values of K between 1 and some cutoff K_{\max} that should be equal to N , the number of elements in the network. However, due to restrictions on computer memory and time, we use $K_{\max} = 30$ if N is larger than 30.

To compute the time evolution of the system, the i th element has an associated Boolean function, or rule table, B_i , which maps the state of all K_i input elements to an output state of either 0 or 1. The fraction of “1” output states is designated as the biasing parameter p . Because of the symmetry of p around 0.5, we can choose $0.5 \leq p \leq 1.0$. The evolution of the system takes place in discrete time steps. At each step, all N elements in the network are updated synchronously. Once the rule table and all the connectivities have been defined, we initialize each element in the network randomly, and then update the network synchronously for 500 time steps to allow transients to die off. We continue updating until we either hit an arbitrarily chosen cutoff or find a fixed or periodic state.

In general, Boolean networks will move into one of three different types of attractors.⁴ The system can fall into a fixed state, a periodic state, or a “disordered” state. A disordered state is characterized by periods that grow exponentially with N , the number of elements in the network. Thus for large N , these states appear to be nonrepeating or aperiodic. Deciding how long a period must be to be termed disordered is arbitrary: There is no clear boundary between the periodic and disordered states. We choose a cutoff of 1500.

In this paper, we characterize the dynamics of networks having three types of distribution functions $P(K)$. In particular, we look at the following distributions:

(1) The delta function distribution, where $P(K)$ is nonzero only for $K = K_{\text{mean}}$.

- (2) The Poisson distribution, given by $P(K) = (x^K e^{-x})/K!$, with $x = K_{\text{mean}}$. If the random value for K_i chosen from this distribution is zero we assign $K_i = 1$, and if the value lies above K_{\max} we set $K_i = K_{\max}$.
- (3) A power law distribution given by $P(K) = AK^{-\beta}$, for $1 \leq K \leq K_{\max}$. Here we chose the exponent β by picking the mean of the distribution to be equal to K_{mean} . The coefficient A is determined by requiring $P(K)$ to be normalized.

III. RESULTS

First, we analytically study an order parameter of these networks: the fraction of elements in a network that remains active after an initial transient. Studying this order parameter allows us to make predictions about the location of the critical point. At this point the network goes from a frozen state to one with a finite fraction of active elements. We also study the system numerically using three different measures. We first classify the types of attractors as a function of bias p and mean connectivity K_{mean} . Next we characterize the networks in terms of the order parameter. Finally, we measure the lengths of periodic orbits. In all three measures, the power law networks show more order.

A. Analytical results

First we look at the fraction u of the network that remains active after an initial transient. Previously, this quantity $u = u(p, K_{\text{mean}})$ was studied in the original Kauffman network as a function of the bias p and the mean connectivity K_{mean} . This quantity was first studied using an annealed approximation.^{17,18} Later, Flyvbjerg¹⁹ found an analytical expression for the critical value of p at which a network with a fixed connectivity for each element would undergo a phase transition from a totally frozen state to one where a finite fraction of the elements are active. This analysis was done in the limit as $N \rightarrow \infty$ (the thermodynamic limit). This critical point is given by

$$p_{\text{crit}} = \frac{1}{2} \left(1 + \sqrt{1 - \frac{2}{K}} \right).$$

We extend Flyvbjerg’s analysis to the case where the connectivity of each element K_i is chosen from a distribution $P(K)$. We find a map that expresses how the “frozen component” grows during a time step as a function of its size at the previous time step. The frozen component at time t is defined as the fraction $s(t)$ of elements in the network that do not change after time t . Thus we are looking for a map $F(s)$ such that $s(t+1) = F(s(t))$. We look at a specific element with K inputs. There are in general $K+1$ ways for this element to be engulfed by the frozen component. We write down these probabilities and then sum all $K+1$ terms to find the total probability that an element will become frozen. For example, the “zeroth” way for this to occur is if all K inputs to the element are frozen. The probability of this occurring is given by $\sum_{K=1}^{\infty} s^K P(K)$, where $P(K)$ is again the distribution of inputs. The Appendix shows the complete calculation.

The final result is given by

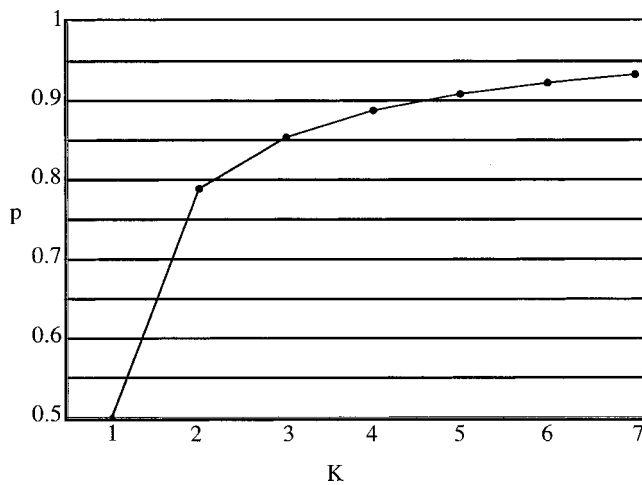


FIG. 2. The theoretically obtained critical value of the bias p as a function of the mean number of inputs K_{mean} . In the limit $N \rightarrow \infty$, networks with values of p above the critical value would have only frozen elements.

$$p_{\text{crit}} = \frac{1}{2} \left(1 + \sqrt{1 - \frac{2}{K_{\text{mean}}}} \right),$$

where K_{mean} is the mean value of K . In the thermodynamic limit, the only quantity the critical point depends on is the average value of K . Figure 2 shows a plot of the critical point as a function of the K_{mean} .

B. Numerical results

We ran simulations of networks with $N = 100$ using three different distributions $P(K)$: a delta function, a Poisson distribution, and a power law, as discussed previously. All data were averaged over 500 trials, each with a different network realization and one initial condition per network. These simulations were written in C²³ and run on Sun workstations and Macintosh G3 processors. We calculated several quantities (after an initial transient of 500 time steps) as a function of the bias p for $K_{\text{mean}} = 2, 4, 6$.

1. Attractor classification

We first studied the types of attractors that exist for these networks as a function of p and K_{mean} . We measured the fraction of networks that become fixed, periodic, and disordered, for all three distributions. Figure 3 shows the fraction of disordered networks as a function of p for $K_{\text{mean}} = 4$ and $K_{\text{mean}} = 6$. Comparing Fig. 3 to Fig. 2 we see that the transition between a zero and nonzero fraction of disordered networks is in good agreement with the theoretically obtained critical point. We also note that in the regime where a significant fraction of networks are disordered, the power law networks show considerably more order. This is particularly apparent for the $K_{\text{mean}} = 4$ graph, in which the power law nets clearly have a larger fraction of ordered solutions.

2. Fraction of active elements

Figure 4 shows the fraction of active elements as a function of p for three values of K_{mean} . We mention that if fixed and periodic behavior can occur for the same value of p and K_{mean} , we see large error bars (which we leave off in the

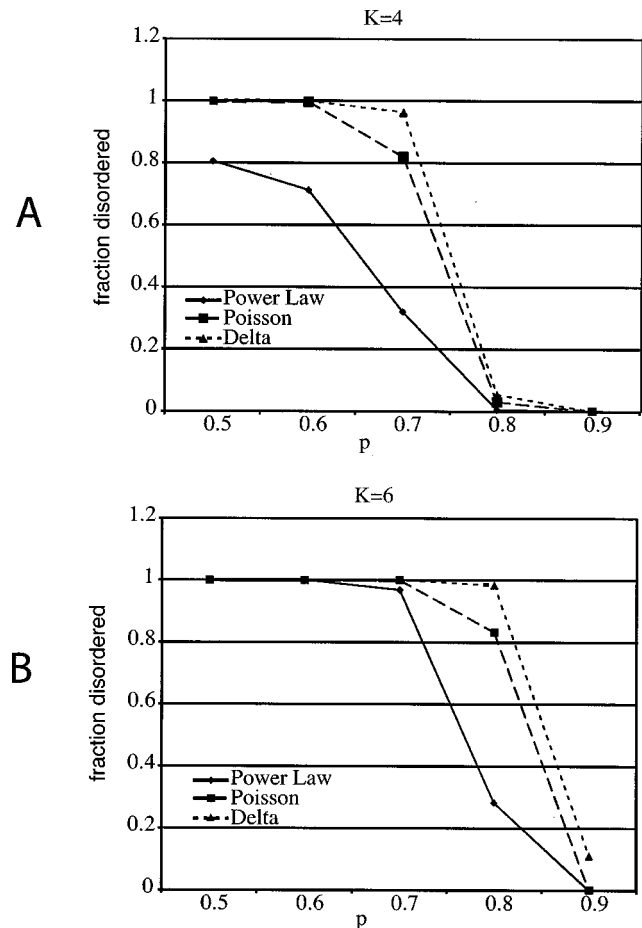


FIG. 3. The fraction of disordered (period > 1500) nets as a function of p for $K = 4$ and 6 and $N = 100$. For $K = 2$, nearly all nets are ordered for any p . Note that for $K = 4$, far fewer power law nets are disordered.

interest of clarity) on the fraction of active elements in a network. For example, at $K_{\text{mean}} = 2$ and $p = 0.5$, where both fixed and periodic solutions can occur, the error bars extend from 0.0 to roughly 0.4. The fact that these attractors tend to coexist near the critical point makes it difficult to find the critical point numerically using this order parameter for finite values of N . However, the rough position seems to be in good agreement with the theory. We note that the power law distributions produce a large fraction of frozen elements even for large K_{mean} and a bias of 0.5. In contrast, the delta function networks have a steep increase in active elements below the critical point.

We can understand the large frozen component in the power law networks in the so-called disordered regime by noting that even if K_{mean} is large, a significant fraction of the elements in the network will have a value of $K = 1$ or $K = 2$. We can write an expression for the lower bound on the size of the frozen component by asking what fraction of the network will be frozen due to elements whose states are independent of their rule tables. This lower bound is given by $s_{\text{LB}} = \sum_{K=1}^{\infty} P(K)p_K$, where $P(K)$ is the distribution of inputs K , and p_K is the probability of an element being independent of all K of its inputs. We can analytically compute this lower bound for the power law distribution as a function of the exponent β . As K_{mean} becomes large, we expect the

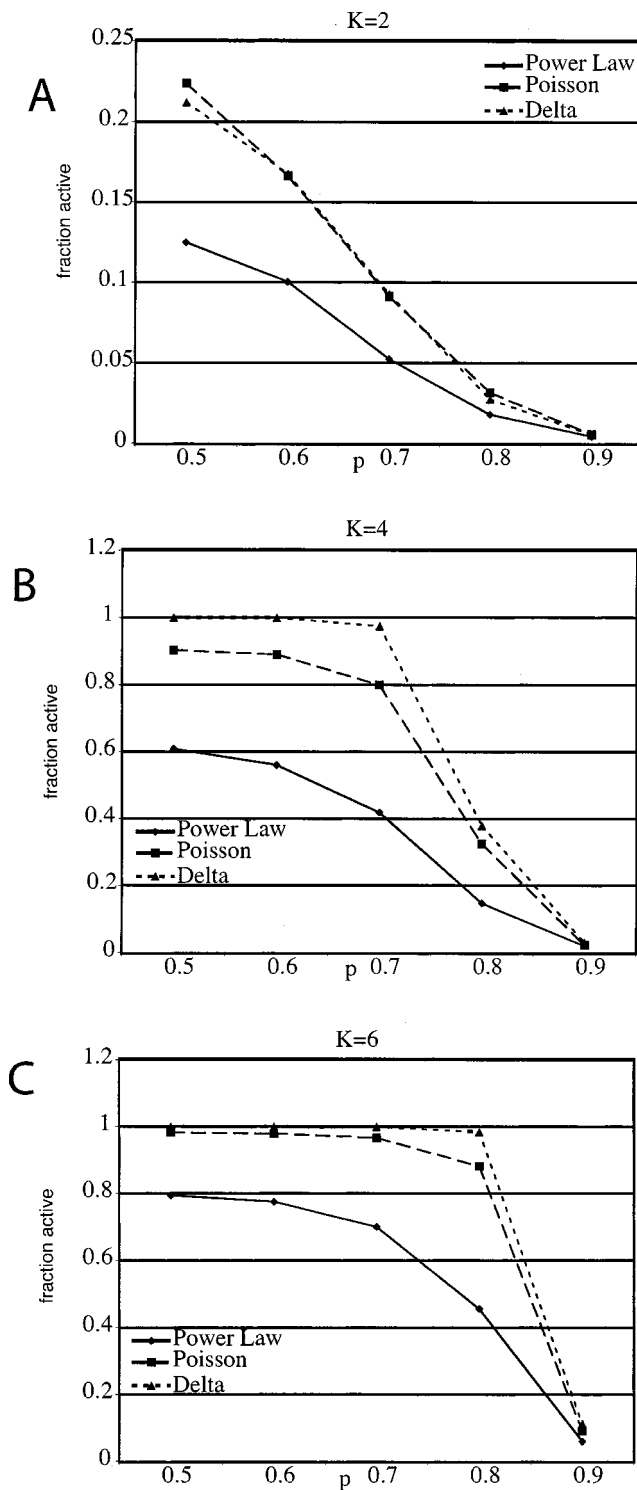


FIG. 4. The fraction of active, or unfrozen, elements in a network as a function of bias p for $K=2, 4,$ and 6 and $N=100$. Note the significant fraction of frozen elements in the power law nets for $p=0.5$ and $K=4$ and $K=6$.

actual steady state fraction of frozen elements to approach this lower bound. Figure 5 illustrates this point.

3. Length of period

Finally, we measured the average period length of periodic networks as a function of the bias p for those values of

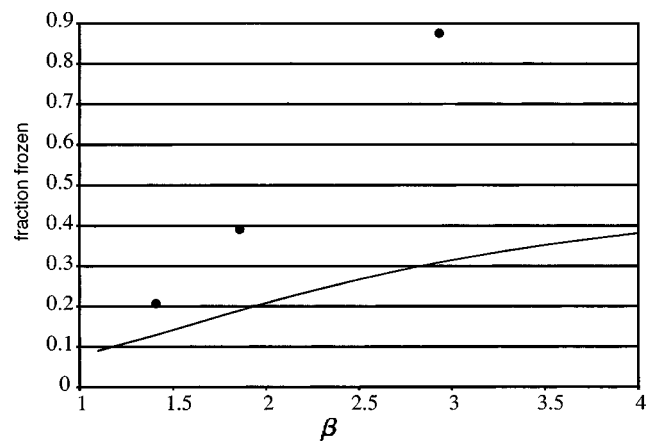


FIG. 5. The lower bound of the fraction of frozen elements in power law nets as a function of the exponent β in the power law with a bias $p=0.5$. This lower bound is found by considering how many elements will be independent of all K inputs. For comparison, we place a dot at the values of β that correspond to the three values of K_{mean} that we use in our simulations. For $K_{\text{mean}}=2$, the frozen component is significantly larger than the lower bound, but as K_{mean} increases (β decreases), the frozen component remains closer to the lower bound.

p and K_{mean} that produce periodic dynamics. We again observe that the networks with power law distributions appear more ordered than the Poisson and delta functions distributions. See Table I. The delta function and Poisson networks have disordered dynamics at $p=0.5$ and 0.6 . The power law networks have much shorter average periods in the periodic regime.

IV. DISCUSSION

This study was done to investigate the relationship between topology and dynamics in biochemical networks. We have cited three pieces of recent experimental evidence that suggest that biochemical networks have broad distributions in the number of inputs and outputs. The possibility that these networks may have broad distributions has been suggested previously. For example, Somogyi and others²⁰ have proposed that multigenic (elements with many inputs and few outputs) and pleiotropic (elements with few inputs and many outputs) elements may be common and important organizational themes in real cellular networks. Figure 6 schematically illustrates these two types of organizational themes, as well as two other types: “simple node” elements (few inputs and few outputs) and “super node” elements (many inputs and many outputs). The mammalian cell cycle provides several examples of these strategies.¹¹ RPaseII (8 inputs, 2 outputs) is a multigenic element; ATM (2 inputs, 5 outputs), is a pleiotropic element; Max (one input, one output) is a simple node, while p53 (26 inputs, 14 outputs) is a

TABLE I. Average period for $k=4$.

Bias p	0.5	0.6	0.7	0.8	0.9
Delta function	388	53.2	3.54
Poisson	308	54.0	3.65
Power law	268	185	133	16.3	3.52

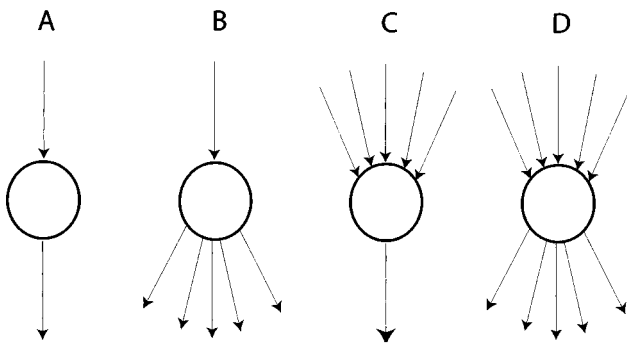


FIG. 6. This is a schematic of the four types of organizations. (a) “Simple node.” A node with few inputs and few outputs. Max is an example from the Kohn map. (b) “Pleiotropic node.” A node with few inputs and many outputs. ATM is an example from the Kohn map. (c) “Multigenic node.” A node with many inputs and few outputs. RPselII is an example from the Kohn map. (d) “Super node.” A node with many inputs and many outputs. p53 is an example from the Kohn map.

super node. Similar examples can be found in gene regulation networks. For example, some transcription factors such as the zinc-finger protein Sp1 in mammals control the expression of more than 300 genes.²⁴ Others, such as the lac repressor in *E. coli* bacteria, control only a single gene.⁸ This variety of organizational strategies within a network is only possible if there is a broad distribution of connectivity.

Motivated by evidence for broad distributions in real biochemical networks, we studied Boolean networks with delta function, Poisson, and power law distributions in a number of inputs to see how these topological modifications affect dynamics of the network. We first analytically showed that in the thermodynamic limit, the critical point does not change with the addition of the distribution in K . This calculation was done by extending the method of Flyvbjerg.¹⁹

We next studied finite delta function, Poisson, and power law networks numerically. We characterized these three types of networks using three different measures: type of attractors, fraction of active elements, and length of period. We measured these three quantities as a function of the interaction bias p and the average connectivity K . We found that for all three measures, the power law nets show considerably more order than the delta function networks that had been studied previously.

One plausible mechanism that may contribute to this ordered behavior is the following. The power law distribution not only is characterized by a heavy tail; it also produces values of K near 1 with high probability, even if the mean value of K is large. These elements with few inputs are much more likely to be frozen, and these frozen elements reduce the size of the network that is still active by a significant fraction. These elements effectively reduce the mean value of K for the network; even if a particular element has a large number of inputs, a significant fraction of those inputs will be frozen. For example, for $K=4$ and $p=0.7$, we measured the effective mean K (the average number of active inputs to active elements) for all three distributions. We found that the delta function, Poisson, and power law distributions had effective mean values of K of 3.0, 2.7, and 2.1. Thus, the order that is introduced by a large number of small K elements

may outweigh the disorder produced by a few elements with very large K .

Our numerical studies show that finite networks with broad, scale-free distributions in connectivity can show more order than networks with sharply peaked distributions. For a given number of elements N and a given mean connectivity K_{mean} , a network randomly selected using a scale-free distribution of K is more likely to be ordered than one selected using a tight distribution. Perhaps this fact is one reason why real biological networks exhibit broad distributions in connectivity.

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APPENDIX: DERIVATION OF CRITICAL POINT

For a distribution of inputs $P(K)$, the “zeroth” way for an element to become frozen is if all K inputs are frozen. This probability is given by $\sum_{K=1}^{\infty} s^K P(K)$. The next way for an element to become frozen is if all but one of the inputs are frozen and if the state of this element is independent of the remaining active element. The probability of this occurring is given by $\sum_{K=1}^{\infty} K s^{K-1} (1-s) p_1 P(K)$, where p_1 is the probability that an element with one input is independent of that input. In general, p_k is defined as the probability that an element with k inputs is independent of all k inputs. This probability will depend on the bias of the network.

We can continue to write down these terms using similar reasoning. Thus the k th term in the sum will be given by

$$\sum_{K=1}^{\infty} \left(\frac{K!}{k!(K-k)!} \right) s^{K-k} (1-s)^k p_k P(K),$$

where p_0 is defined to be 1.

Summing all $K+1$ terms gives us

$$s(t+1) = \sum_{K=1}^{\infty} \sum_{k=0}^K \left(\frac{K!}{k!(K-k)!} \right) s^{K-k} (1-s)^k p_k P(K). \tag{A1}$$

This is the expression of the return map. Now we let $u = 1-s$, where u is the fraction of elements that are unfrozen. Then we have

$$u(t+1) = 1 - \sum_{K=1}^{\infty} \sum_{k=0}^K \left(\frac{K!}{k!(K-k)!} \right) \times (1-u)^{K-k} u^k p_k P(K). \tag{A2}$$

Clearly $u = u^* = 0$ is a fixed point. Now we expand around this fixed point to study its stability. To find the critical point, we look at when this fixed point will go unstable.

Let $u = u^* + \delta$ with δ a small number. Then we can re-write Eq. (A2), keeping only the zeroth- and first-order terms. The $k=0$ term in the sum provides one zeroth-order term and one first-order term. The $k=1$ term in the sum provides one first-order term. Adding these we have

$$\delta(t+1) = 1 - \left\{ 1 + \sum_{K=1}^{\infty} -K \delta p_0 P(K) + \sum_{K=1}^{\infty} K \delta p_1 P(K) \right\}. \tag{A3}$$

Now, we know $p_0 = 1$, so we must determine p_1 .

Recall that p_1 is the probability that an element is independent of its only active input. If all but one input to an element are frozen, then the effective rule table for that element has only two entries, one for each state of the input element. For the state of the element to be independent of the input, we need both of these entries in its rule table to have the same value. This occurs with probability $p^2 + (1-p)^2$, where p is the bias of the network.

We can now write

$$\delta(t+1) = \delta \left(\sum_{K=1}^{\infty} KP(K) - p_1 \sum_{K=1}^{\infty} KP(K) \right)$$

or

$$\delta(t+1) = \delta(\langle K \rangle - p_1 \langle K \rangle) = \delta \langle K \rangle (1 - p^2 + (1-p)^2). \tag{A4}$$

The fixed point goes unstable when the coefficient of the linear term has absolute value larger than 1. This occurs at

$$p_{\text{crit}} = \frac{1}{2} \left(1 + \sqrt{1 - \frac{2}{K_{\text{mean}}}} \right). \tag{A5}$$

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