Genetic and logic networks with the signal-inhibitor-activator structure are dynamically robust*

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Received February 16, 2006; revised June 16, 2006

Abstract The proteins, DNA and RNA interaction networks govern various biological functions in living cells, these networks should be dynamically robust in the intracellular and environmental fluctuations. Here, we use Boolean network to study the robust structure of both genetic and logic networks. First, SOS network in bacteria *E. coli*, which regulates cell survival and repair after DNA damage, is shown to be dynamically robust. Comparing with cell cycle network in budding yeast and flagella network in *E. coli*, we find the signal-inhibitor-activator (SIA) structure in transcription regulatory networks. Second, under the dynamical rule that inhibition is much stronger than activation, we have searched 3-node non-self-loop logical networks that are dynamically robust, and that if the attractive basin of a final attractor is as large as seven, and the final attractor has only one active node, then the active node acts as inhibitor, and the SIA and signal-inhibitor (SI) structures are fundamental architectures of robust networks. SIA and SI networks with dynamic robustness against environment uncertainties may be selected and maintained over the course of evolution, rather than blind trial-error testing and being an accidental consequence of particular evolutionary history. SIA network can perform a more complex process than SI network, and SIA might be used to design robust artificial genetic network. Our results provide dynamical support for why the inhibitors and SIA/SI structures are frequently employed in cellular regulatory networks.

Keywords: genetic network, signal-inhibitor-activator (SIA) structure, Boolean network model, dynamical robust.

Through the dynamical processes of interaction networks between proteins, DNA and RNA, the living cells execute various biological functions to respond internal and external changes. Recently, progress in molecular biology and high-through biological technology has revealed more detailed knowledge about the protein interaction and transcription regulatory networks. The major challenge is to find the basic building blocks and the fundamental architectures of transcription regulatory networks, and to highlight the relationship between structure and cellular dynamics or functions, then to reveal the design principle of regulatory networks.

Previous studies have mostly concentrated on the topological aspects of the networks, such as the scale-free distribution, the motifs and modules of networks[1, 2]. The living cells accomplish biological functions and responses to the change of environment by activating or expressing different kinds of proteins; these are dynamical processes. Now the molecular details are lack to build an exact dynamical model to simulate the biological processes and provide experiment-testable predictions. In the absence of information about the kinetic constants and molecular details, it is better to use a simple dynamical model, which ignores molecular details, to study the basic structure of regulatory networks[3, 4]. We had used Boolean network model to study cell-cycle regulatory network in budding yeast, and found that yeast cell-cycle network is robustly designed, the G1 state is a global attractor of the dynamics, and the cell-cycle process is a globally attracting trajectory of the dynamics[5].

The biological systems should be robust to function in the complex and uncertain environments. To be more robust may mean to be more evolvable, and thus easier to survive. Is the cell cycle network unique to behave robustly? Are there other regulatory networks that also have the global robustness? If there are, what is the fundamental structure of these networks? Here, we address the questions in dynamical robust view by Boolean network model simulation. We first study whether SOS network in *E. coli* is dynamically robust, and then try to find a roust structure in cellular transcription regulatory networks. Second, we take dynamical robustness as a criterion to find the fundamental architectures from 3 and 4 nodes non-self-loop logic networks. Finally, we discuss the applications and evolution of robust network structure.

* Supported by the National Program on Key Basic Research Projects (Grant No. 2003CB715900)

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1 Transcription regulatory networks with dynamical robustness and the signal-inhibitor-activator structure

1.1 The SOS network in *E. coli* is dynamically robust.

In this section, we analyze the dynamical properties of SOS network in bacteria *E. coli* by Boolean network model, using the dynamical rule that inhibition is much stronger than activation, which is always true in many bacterial regulatory networks. The SOS network in *E. coli*, which regulates cell survival and repair after DNA damage, involves the inhibitor/activator system of LexA and RecA, and more than 30 genes directly regulated by LexA and RecA. UV DNA damage causes single-stranded DNA (ssDNA). Upon binding to ssDNA, the RecA protein is activated (RecA*) and serves as a coprotease for the LexA protein. When the concentration of LexA protein diminishes, the genes normally suppressed by LexA are more frequently transcribed, such as umuDC and ssb (single strand binding protein gene) that mediate DNA protective responses[6,7]. We start from the complex SOS network in Gardner et al.[6], and combine the DNA repair proteins into UmuDC and SSB nodes, then obtain a simplified 6-node network shown in the inset map of Fig. 1. There are ssDNA, RecA, inhibitor LexA, sigma70, UmuDC, and SSB in simplified SOS network, where ssDNA represents single-stranded DNA, RecA represents activated RecA (RecA*), sigma 70 as σ70 factor.

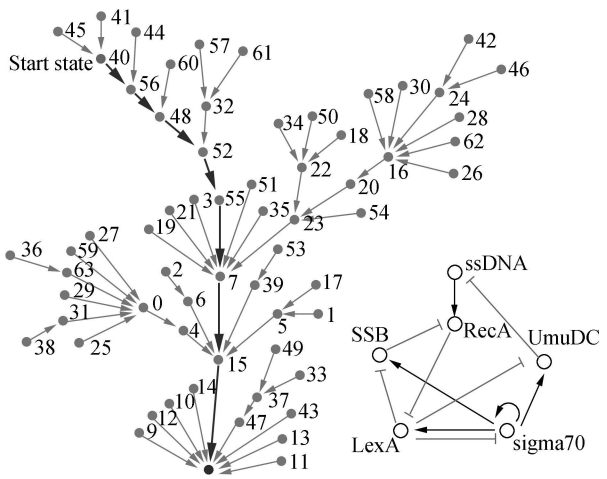

Fig. 1. Simplified SOS network in *E. coli* (inset map) and its dynamical trajectories. Each state or node in the trajectory map is noted by its decimal number. The biological trajectory colored in black starts from the # 40 Start state with single-stranded DNA and activated LexA, and evolves to the stable # 8 attractor with active LexA. In SOS network, "T"-line represents inhibition, arrow denotes activation.

A Boolean network model treats the nodes and arrows as logic-like operations. Each node i has only two states, $S_i=0$ and $S_i=1$, representing the inactive and active states of the protein, respectively. The time step is also treated as discrete step. The protein (node) states in the next time step are determined by the protein (node) states in the present time step via the following rule:

$$S_i(t+1)=\begin{cases}1, & \sum_j a_{ij}S_j(t)>0\\ 0, & \sum_j a_{ij}S_j(t)<0.\\ S_i(t), & \sum_j a_{ij}S_j(t)=0\end{cases} \quad (1)$$

We take the dynamical rule that inhibition is much stronger than activation, which is always true in many bacterial regulatory networks, and so we set $a_{ij}=1$ for an activation arrow from protein j to protein i and $a_{ij}=-\infty$ for an inhibition arrow from j to i. In the simulation, we settle the positive loop of σ70 by giving a constant house keeping activation, so once the lexA is turned off, the σ70 will be activated and begin to transcribe umuDC, ssb and lexA genes.

We first simulate the biological process of SOS network. Starting from the state with single-stranded DNA and active inhibitor LexA (decimal number = 40), ssDNA triggers and activates the signal protein RecA. After active RecA suppresses LexA at the 3rd step (#48), the σ70 factors begin to transcribe and translate UmuDC and SSB to repair DNA at the fifth step (#55). The increasing LexA suppresses UmuDC and SSB again, and the system evolves step by step to a final stable state with active LexA, which are listed in Table 1. This temporal trajectory is consistent with the experimental data and gene expression data[7–9]. The attractor state is {ssDNA, RecA, LexA, σ70, UmuDC, SSB} = {0, 0, 1, 0, 0, 0}, with only active LexA (#8), and we name it active LexA state.

Table 1. The biological trajectory of the simplified SOS network

Step	ssDNA	RecA	LexA	σ70	UmuDC	SSB	Decimal #
1	1	0	1	0	0	0	40
2	1	1	1	0	0	0	56
3	1	1	0	0	0	0	48
4	1	1	0	1	0	0	52
5	1	1	0	1	1	1	55
6	0	0	0	1	1	1	7
7	0	0	1	1	1	1	15
8	0	0	1	0	0	0	8

There are $2^6 = 64$ states in SOS network. Evolving from each state, we obtain all the dynamic trajectories of SOS network shown in Fig. 1, where each node represents a possible state noted with its decimal number, and the black line denotes biological trajectory listed in Table 1. The #8 state with active LexA state is the only global attractor attracting all the 64 initial states. When RecA suppresses active LexA, σ70 can trigger DNA repair. Once LexA is active, it will suppress UmuDC and SSB, and push the system to evolve to the #8 attractor state with active LexA.

There are two major trajectories in the SOS network, starting from #40 and #42. The difference between the two trajectories is whether UmuDC proteins are activated at the start. If UmuDC proteins are not activatd at the start (start from #40), this is the biological trajectory. The ssDNA will activate RecA and cause the SOS response; the ssDNA will be repaired at the end by the activation of UmuDC proteins. However, if UmuDC proteins are activated at the start (from #42), the activation of UmuDC proteins will repair the ssDNA at the start but the ssDNA still activates RecA and causes the following SOS response. Thus, the difference between #56 state (biological trajectory) and #24 state is only the activation of ssDNA at the beginning steps, and so do the #48 and #16, #52 and #20, #55 and #23.

1.2 More transcription regulatory networks with dynamical robustness and the signal-inhibitor-activator structure

The robust yeast cell-cycle network is constituted by signal proteins, activator, inhibitors, and DNA and spindle checkpoints[5, 10]. Whi5, Sic1, Cdc20 and Cdh1 are inhibition or repression proteins in the cell-cycle process; the inhibitor protein can provide a threshold at the start of the process and repress the signal and activator at the end of the process. For example, Sic1 provides a threshold for cyclin Clb5, 6 to pass G1-S phase transition, while Cdc20 and Cdh1 degrade cyclin Clb1, 2 to let the cell exit from mitosis and evolve to stable G1 state with active inhibitor Cdh1. There are two kinds of repressors: one kind provides threshold for signal to avoid random noise triggering the cellular process, like Sic1 and Cdh1/APC in G1/S transition of yeast cell-cycle, and the other kind suppresses the activator to an inactive level at the end of the cellular process to make the system stay at a stable state, like Cdc20/APC and Cdh1/APC in M/G1 transition of yeast cell-cycle.

In the flagella network of *E. coli*, there are three classes of gene transcription for flagellar assembly. The master regulator FlhDC turns on class II genes, including FliA and FlgM. And inhibitor FlgM is used as checkpoint to block FliA. Only when basal body-hook structures are completed and the FlgM is transformed out of cell, can FliA start the 3rd class gene transcription including FlgM[11, 12]. Therefore, FlhDC acts as signal, FlgM as inhibitor, and FliA as activator. The dynamics of flagella network is complex for the protein degradation and FlgM transformation, and flagella network is dynamically robust against state change under a certain dynamical rule (results omitted).

In Table 2, all the three cellular regulatory networks have inhibitors, and a signal-inhibitor-activator (SIA) structure is outlined: the signal node activates activator; inhibitor represses signal node or activator. Kirscher and Gerhart insisted that it is easy to find repressor in the genetic regulator networks, and the regulation of cellular processes is imposed chiefly by inhibitions[13]. Why do cellular networks utilize inhibitors? Do inhibitor and SIA structure benefit dynamical robustness of networks? Fig. 2(b) shows a SIA structure network is dynamically robust. More evidence will be shown in the following sections.

Table 2. The signal-inhibitor-activator (SIA) structure in cellular transcription regulatory networks

	Signal nodes	Repressors or inhibitors	Activators
SOS in *E. coli*	RecA*	LexA	σ70
Flagella in *E. coli*	FlhDC	FlgM	FliA
Cell cycle in yeast	Cln3 Cln2/Clb5	Whi5 Sic1/Cdh1, Cdc20/Cdh1	SBF Mcm1

2 The 3 and 4 nodes logic networks with dynamical robustness

In this section, we take the dynamical robustness as a criterion to study the possible structures of robust logic networks by Boolean network model. The dynamical robustness is defined as that the network has one global attractor with the biggest attractive basin. We search all possible 3-node networks and part of 4-node networks without self-loops. There are 3 possible interactions from node A to node B, inhibition, activation or none. All the possible structures of 3-node networks without self-loop are $3^{9-3} = 729$; all the possible structures of 4-node networks without self-loop are $3^{16-4} = 531441$. Each node in networks has two states. 0 and 1 represent inactive and active states, respectively, and so there

are 8 possible states for 3-node network and 16 states for 4-node network. The number of all possible structures of 3 and 4 nodes networks is not too large to be completely enumerated, and the dynamical property of the small nodes networks is easier to obtain by Boolean network model. Thus, we can find the relationship between the network structure and its dynamics, where dynamics is related to the biological function of networks, and long dynamical trajectory can execute a complex biological process.

We focus on the dynamical rules that inhibition is much stronger than activation (rule 1), and discuss lately the rule that inhibition is equal to activation (rule 2). We only discuss the networks without self-loops, ignore the negative self-loops and positive self-loops, where positive self-loop cannot trigger the node state from inactive to active.

2.1 The 3-node networks with dynamical robustness under the rule that inhibition is much stronger than activation

We search for all possible 3-node networks without self-loop (729 networks) under the rule that inhibition is much stronger than activation; we find 28 un-isomorphic robust networks with the biggest attractor basin equal to 7 (BB=7). There are 8 states in a 3- node network, and the inactive state (0, 0, 0) evolves to itself in Boolean network model, so the biggest attracting basin is 7 for non-(000) attractors. Classifying these 28 networks by their final attractor states, there are 9 networks whose final attractor state has only one active node—(100) attractor type, 14 networks whose final attractor state has two active nodes—(110) attractor type, and 5 networks whose final attractor state has all 3 active nodes—(111) attractor type.

First, we study nine (100) attractor type networks shown in Fig. 2(a). We find that node 2 represses other nodes in all 9 networks, and node 1 and node 3 will activate node 2 directly or indirectly. Under rule 1, once node 2 is active, the other nodes will become inactive in the next time step. We define node 2 as inhibitor or repressor. So in Fig. 2(a) node 2 acts as inhibitor, node 1 and 3 as signal or activator node, and the final attractor state is (0, 1, 0). The dynamical trajectory of network N1-1 is shown in Fig. 2 (b). In the trajectory from (1, 0, 0), the inhibitor is activated at the 3rd step and then suppresses signal node and activator at the 4th step to (0, 1, 0) attractor. The states with inactive inhibitor have relatively long steps process. Fig. 2(c) shows the dynamical trajectory of network N1-5, where inhibitor represses both signal and activator.

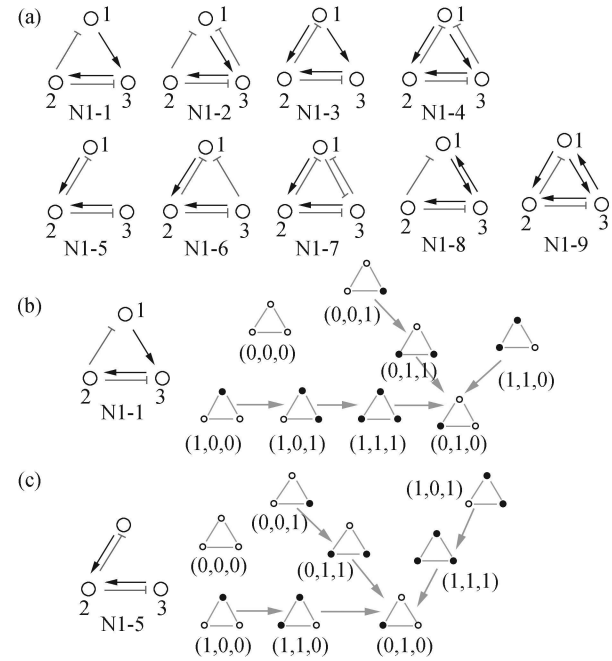

Fig. 2. Nine robust (100) attractor-type 3-node networks with BB=7 under the rule that inhibition is much stronger than activation. (a) Node 2 acts as inhibitor in the (100) attractor type networks, and networks 1, 2, 3, 4, 8, 9 share SIA structure, networks 5, 6, 7 share SI structure; (b) the root network N1-1 and its dynamical trajectories; (c) the root network N1-5 and its dynamical trajectories.

These 9 networks can be generated from two basic root networks, network N1-1 and N1-5, by adding inhibition ("T"-line) or activation (arrow) interactions shown in Fig. 3. The 6 networks on the root network N1-1 have the signal-inhibitor-activator (SIA) structure. The other 3 networks have signal-inhibitor (SI) structure. Our results provide evidence that the fundamental signal-inhibitor architecture provides dynamical robustness, so it may be produced and maintained over the course of evolution.

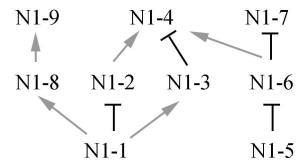

Fig. 3. The (100) network structure relation trees under the rule that inhibition is much stronger than activation. The "T"-line from network N1-1 to N1-2 represents that N1-2 can be generated from N1-1 by adding an inhibition action. The arrow denotes adding activation action, and all the 6 networks on network N1-1 share the SIA structure, networks 5, 6, 7 on network N1-5 share SI structure.

On the other hand, SIA structure network N1-1 can generate 9 possible networks without self-loop, including the 6 network with BB=7 in Fig. 2(a). The other 3 networks have inhibition from node 1 to node 2 whose BB is smaller than 7 (results omitted).

Then we provide a proof for the above results. Using Boolean network model with the dynamical rule that inhibition is much stronger than activation, if the biggest attractor basin of the non-self-loop 3-node network is 7 and the final attractor is (0, 1, 0), then the network should have the following topological properties:

(i) The node 2 should inhibit all other nodes directly. So node 2 works as an inhibitor. Otherwise, the state (1, 1, 0) or (0, 1, 1) should not evolve to final attractor (0, 1, 0).

(ii) Other nodes should activate the inhibitor node directly or indirectly. Otherwise, the state (1, 0, 0) or (0, 0, 1) should not evolve to final attractor (0, 1, 0).

There are 14 (110) attractor type networks and 5 (111) attractor type networks, whose final attractor state has 2 or 3 active nodes as shown in Fig. 4 (a) or Fig. 4(b), respectively.

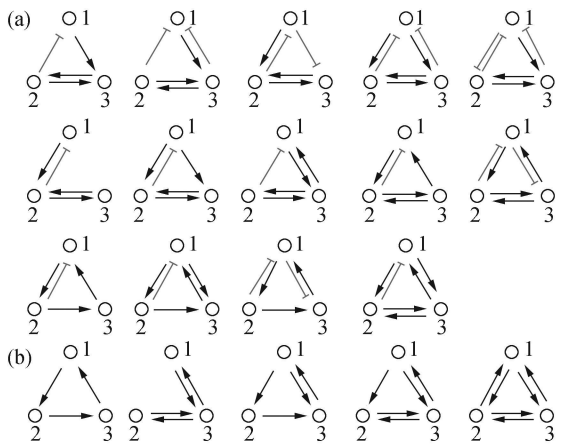

Fig. 4. Robust (110) and (111) attractor-type 3-node networks with BB=7 under the rule that inhibition is much stronger than activation. (a) 14 networks with two active nodes (110) attractor type. (b) 5 networks with 3 active nodes (111) attractor type. No inhibitor node appears in networks.

In (110) attractor type networks, the activation arrows appear more frequently with fewer inhibitions, and there is not any inhibitor-like node. We have arranged the state of the biggest attractor as (0, 1, 1) with active nodes 2 and 3. Similar to (100) type attractor, we can find and prove that the networks in Fig. 4(a) should have direct or indirect activation between nodes 2 and 3, while node 2 or 3 should repress node 1 to ensure that (0, 1, 1) is the biggest attractor.

In (111) attractor type networks in Fig. 4(b), there are activation and no inhibition, and each node should activate other nodes directly or indirectly to ensure that (111) is the biggest attractor.

The biggest attractor basin BB=7 for non-self-loop 3-node network is a strong constrained condition. Further discussion about n-node network with $BB=2^n-1$ can be found in Ref.[14].

2.2 The 4-node networks with dynamical robustness under the rule that inhibition is much stronger than activation

Adding a node as a functioning node of activator, we search for 4-node networks without self-loop under the two different rules, and under the constrained conditions that: signal node (the node 1) activates activator (the node 3), inhibitor (the node2) suppresses activator, activator activates function factor (the node 4); and (1, 1, 0, 0) state evolves to (0, 1, 0, 0).

Under the rule that inhibition is much stronger than activation, we find 183 un-isomorphic networks with BB$>$11. We analyze their connection matrix, and obtain the probability of each element value in the matrix (detail omitted). If the threshold is set to be 0.6, we obtain the network as shown in Fig. 5(a). This network also has a (0, 1, 0, 0) attractor with BB=15 as shown in Fig. 5(b). We can find the similar property to 3-node networks that once the inhibitor node 2 is active, other active nodes will be suppressed in one step, and all states with active inhibitor will evolve to attractor just in one step. Only the states with inactive inhibitor have a relatively long steps process to accomplish the complex biological process.

2.3 The 3 and 4 nodes networks with dynamical robustness under the rule that inhibition is equal to activation

Our previous study on yeast cell-cycle network shows that the dynamical rule with equal inhibition and activation (rule 2) produces similar results to a stronger inhibition rule (rule 1)[5]. Then we study 3-node non-self-loop networks under the rule that inhibition is equal to activation, and there are 20 un-iso-

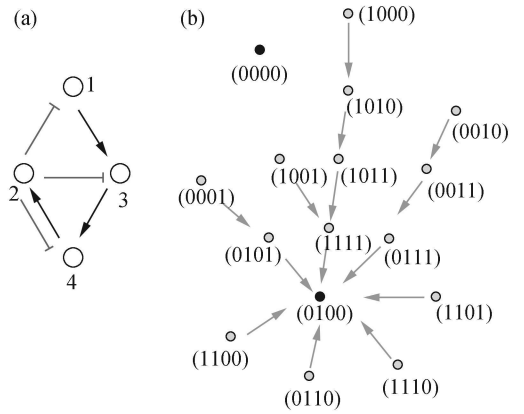

Fig. 5. A robust 4-node network (a) and its dynamical trajectories (b) under the rule that inhibition is much stronger than activation, where node 2 is an inhibitor and (0, 1, 0, 0) state is a global attractor with BB= 15.

morphic networks with BB=7. Classified by the type of final attractor state, there are 8 networks in Fig. 6 (a) whose final attractor state has only one activated node (0, 1, 0), and 7 networks have active nodes in the attractors functioning as inhibitor or repressor except network N2-8. There are 7 networks with BB= 7 and (110) attractor type whose final attractor state has two active nodes, and 5 networks with BB= 7 and (111) attractor type whose final attractor state has 3 active nodes. We do not list (110) and (111) attractor types networks here.

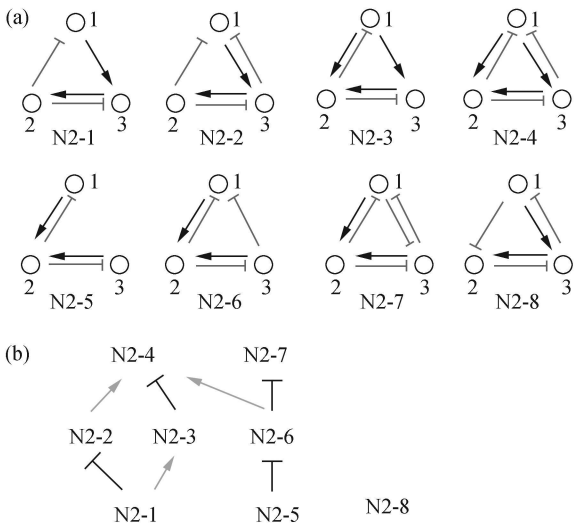

Fig. 6. Robust 3-node networks and their network structure relation trees under the rule that inhibition is equal to activation. (a) Eight 3-node networks with BB= 7 and (100) attractor type, they are the same as networks under rule 1 except N2-8, node 2 acts as inhibitor except in network N2-8; (b) the relation trees, all the 4 networks on the root network N2-1 share the SIA structure.

These 8 networks with the attractor state of (0, 1, 0) constitute a two-root tree in Fig. 6(b). It can be seen that the network N2-8 is alone, and the 4 networks on N2-1 also have the SIA structure. The networks 1—7 share the same structure under rule 1 and rule 2. The network N2-8 is the only one that has no inhibitor and is separated alone from the main structure tree.

Under this equal rule, we also search for 4-node networks without self-loop, and find 161 un-isomorphic networks with BB> 11. If the threshold is set as 0.6, we obtain the network similar to the network in Fig. 5(a) but with an inhibition from node 3 to node 1. This network has one attractor (0, 1, 0, 0) with the biggest attractive basin of 15.

We have also analyzed the 3-node networks with BB= 6 under the above two rules. There are also some network structure trees and several inhibitors, but no global inhibitors and SIA structure.

3 Discussion and conclusion

In this section, we first summarize our results on robust genetic and logic networks, and then discuss the application of SIA/SI structure in cellular regulatory networks. Finally, we infer the evolution history of robust cellular regulatory networks.

Using Boolean network model, we have studied the dynamical robustness of cellular regulatory networks. Our results show that yeast cell-cycle network[5], SOS network and flagella network in *E. coli* are dynamically robust against state fluctuation, and inhibitors and signal-inhibitor-activator (SIA) structure play a crucial role in ensuring network robustness.

Then taking dynamical robustness as a criterion and under the stronger inhibition dynamical rule, we find some structural properties of 3-node non-self-loop logic networks with BB= 7:

(i) Nine (100) attractor type networks are shown in Fig. 2(a), where inhibitor node appears in all nine networks, and SIA and signal-inhibitor (SI) structures are the fundamental framework, where inhibitor node is defined as the node repressing other two nodes.

(ii) There are fourteen (110) attractor type networks in Fig. 4(a) that have inhibitions but no inhibitor-like node. Fig. 4(b) illustrates the five (111) attractor type networks, whose nodes activate each

other without any inhibition.

The 3-node networks are the minimal logical representations of cellular transcription regulatory networks. The SIA and SI structure (100) networks in Fig. 2(a) are dynamically robust with the active node in the final attractor as inhibitor. These networks are convenient to be employed in cellular regulatory networks. The active inhibitor represses other nodes, and only when the inhibitor is repressed can the signal trigger activator to accomplish the long process. For example, RecA in SOS network suppresses inhibitor LexA at the beginning of DNA repair process as shown in Fig. 1. After the completion of DNA repair, the re-activated LexA will push the systems back to stablize attractor with active LexA. In yeast cell-cycle network, the cell size signal triggers Cln1, 2, then Cln1, 2 degrades Sic1 and Cdh1/APC to start a DNA replication process, lately re-activated inhibitors Cdh1/APC, Cdc20/APC and Sic1 will push the cell to exit from mitosis. One cell is divided into two, then the system evolves back to stable G1 attractor states with active inhibitors Cdh1/APC and Sic1[5].

However, the network with (110) attractor in Fig. 4(a) may be difficult to use in cellular networks with the 2 active nodes state in the final attractor. The (111) networks in Fig. 4(b) resemble a "ring" signal transduction pathway with a series of activations, but the active kinases in cellular signal transduction pathway become inactive gradually.

Elowitz and Leibler had constituted a three-inhibition circuit to perform oscillation in *E. coli*, but it is sensitive to noise[15]. Inhibitors and SIA structure networks may be useful for designing robust artificial genetic circuits against protein abundance noise. In cellular genetic regulatory networks, inhibitor and repressor proteins appear frequently, and the regulation of cellular processes is imposed chiefly by inhibitions, and these inhibitions are often relieved by another inhibition, producing activation[13]. Based on SIA structure, introducing the inhibitor-function node that represses others, we may establish robust genetic networks. The designed robust genetic networks should take into account the self-loops and molecular details of genetic network, where positive feedbacks always provide a threshold for the signal and a potential irreversible protein state pattern transition[16]. The robust networks with molecular details may be studied by ordinary differential equation (ODE) model to put forward some experiment-testable predictions.

Murray put forward a hypothesis about the evolutionary history of cell-cycle regulatory network: first the inhibitor appeared, then the checkpoint-like inhibition, and then the checkpoints appeared[3,17]. Following Murray, we guess the evolutionary trace of cellular regulatory networks from the dynamic view. First, the earliest and simplest networks may be a two-component system in bacteria and linear signal transduction cascades in eukaryote with signal and activators without inhibitors. The linear signal transduction cascade is that a protein activates a receptor protein, and the receptor activates its following protein. Second, the cells should have evolved to the signal, activators and inhibitors system, and inhibitors and SIA structure may provide dynamical robustness. Finally, checkpoints are introduced to ensure the event order of biological processes, and several signal-inhibitor-activator networks are coupled together with checkpoints to govern more complex processes like cell-cycle process. The evolution may begin from linear cascades with signal and activators, to robust SIA or SI networks with inhibitors, and then to couple several SIA networks by adding checkpoints. These gradually ensure the network dynamical robustness and reliability of cellular event order. During the course of evolution, the dynamical robustness may be used as a design principle to select and refine the genetic regulatory networks. Our further work is to study the large-sized logic networks and their structure robustness.

Robustness is an essential property of biological systems[18,19]. In this paper, the dynamical robustness is defined as that the network has one global attractor with the biggest attractive basin. Using Boolean network model, we have found the robust SIA/SI architectures from both cellular transcription regulatory networks and logic networks. The signal-inhibitor-activator (SIA) structure is that signal node triggers activator; inhibitor represses signal node and activator. Our results provide a support for why the inhibitors and SIA structure are frequently employed in cellular regulatory networks. In the future, we will design and refine robust genetic circuits against external and internal fluctuations based on the robust SIA networks.

Acknowledgements The authors would like to thank Prof. Tang Chao, Prof. Ouyang Qi, Prof. Lai Luhua and Ni

Ming for their helpful discussions.

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