Physics Letters A 374 (2010) 4749–4755

Contents lists available at ScienceDirect

Physics Letters A

www.elsevier.com/locate/pla

Comparison of stationary and oscillatory dynamics described by differential equations and Boolean maps in transcriptional regulatory circuits

Weiming Ye [a,b,1], Pengfei Li [a,1], Xuhui Huang [a], Qinzhi Xia [a], Yuanyuan Mi [a], Runsheng Chen [c,*], Gang Hu [a,b,**]

[a] Department of Physics, Beijing Normal University, Beijing 100875, China
[b] Beijing-Hong Kong-Singapore Joint Center of Nonlinear and Complex Systems, Beijing Normal University Branch, Beijing, China
[c] Bioinformatics Laboratory, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

ARTICLE INFO

Article history:
Received 21 June 2010
Received in revised form 9 September 2010
Accepted 13 September 2010
Available online 8 October 2010
Communicated by C.R. Doering

Keywords:
Ordinary differential equation
Boolean map
Gene regulatory network

ABSTRACT

Exploring the principle and relationship of gene transcriptional regulations (TR) has been becoming a generally researched issue. So far, two major mathematical methods, ordinary differential equation (ODE) method and Boolean map (BM) method have been widely used for these purposes. It is commonly believed that simplified BMs are reasonable approximations of more realistic ODEs, and both methods may reveal qualitatively the same essential features though the dynamical details of both systems may show some differences. In this Letter we exhaustively enumerated all the 3-gene networks and many autonomous randomly constructed TR networks with more genes by using both the ODE and BM methods. In comparison we found that both methods provide practically identical results in most of cases of steady solutions. However, to our great surprise, most of network structures showing periodic cycles with the BM method possess only stationary states in ODE descriptions. These observations strongly suggest that many periodic oscillations and other complicated oscillatory states revealed by the BM rule may be related to the computational errors of variable and time discretizations and rarely have correspondence in realistic biology transcriptional regulatory circuits.

1. Introduction

Since the genomes of many organisms have been sequenced, the rules of gene expressions have attracted lots of focuses. Exploring the principle and relationship of gene transcriptional regulations has been becoming a generally researched issue. A great variety of works have been done in this field, especially in the recent decade [1–19].

In 2002, U. Alon et al. discovered a kind of over-represented blocks in biological networks [20] and many other networks explored the similar behaviors [21]. They found that these small blocks play a very important role in real gene networks and named them motifs. Since then, biologists have paid much attention to those simple transcriptional regulatory (TR) circuits, in both theoretical and experimental researches. Scientists make efforts in two different ways. On one hand, experimentalists search this kind of small networks *in vivo*, or manage to synthesize simple TR circuits *in vitro*, to acquire specific functions, such as toggle switches, periodic oscillations, etc. [22–25]. Elowitz and Leibler designed and experimentally constructed an autonomous TR circuit with three mutually transcriptional repressors showing oscillatory expression variation [24]. On the other hand, a large number of theoretical works have also been done to study the intrinsic dynamics of simple TR circuits [26–32].

Up to date, there are two major methods widely applied for theoretically studying the dynamics of transcriptional regulatory circuits. In certain experimental situations, the protein concentrations and the reaction rates can be quantified so that we can use ordinary differential equations (ODEs) to model the TR circuits [33–37]. However, in most circumstances, the genetic circuits cannot be specified by ODE because of the lack of necessary data. So, the Boolean map (BM) is often used as the time and variable discretized version of the ODE method to describe simple genetic circuits [38–43]. By using these logic rules, some qualitative conclusions are expected to be predictable. It has been generally accepted that the ODE method may more accurately describe the network dynamics, while the BM method may qualitatively catch the essences of the ODE dynamics, but may deviate quantitatively from the actual dynamics in some detail. More comprehensive comparisons have been made by a number of papers. In [38] the authors numerically compared the stationary solutions of both ODEs and BMs for two mutually regulated genes. They found that

* Corresponding author. Tel.: +86 10 64888543; fax: +86 10 64871293.
** Corresponding author at: Department of Physics, Beijing Normal University, Beijing 100875, China. Tel.:+86 10 58807855; fax: +86 10 58800567.
E-mail addresses: crs@sun5.ibp.ac.cn (R. Chen), ganghu@bnu.edu.cn (G. Hu).
[1] Weiming Ye and Pengfei Li contributed equally to this work.

0375-9601/$ – see front matter
doi:10.1016/j.physleta.2010.09.066

the steady states of BMs are preserved in the corresponding ODEs (called regular steady points, RSP) while it is possible that ODEs may have some stable steady states around switching thresholds which the corresponding BMs do not have (called singular steady point, SSP). And similar conclusions have been analytically derived in [44–46] by the comparisons between BMs and their piecewise linear ODE versions. However, to our knowledge, no systematical comparison between these two major methods with certain numeric exhaustive explorations, including nonstationary solutions, has been made. And there has been no answer to the question of crucial importance: *is it possible that in certain cases the two methods give essentially different results which are significant in biology aspect*. Another question follows if the answer to the first question is yes: *when these essential differences occur*.

In 2006, Nochomovitz et al. made an exhaustive enumeration of all autonomous 3-gene and 4-gene circuits using two kinds of Boolean rules [47]. Several interesting conclusions were drawn from their work. One of the most important results is that dynamical periodic oscillatory states can be generated from atypically many networks. However, so far there have been really seldom biological evidences revealing autonomous spontaneous oscillations in few-gene circuits. The observations in [47] have raised an interesting problem whether these atypically many oscillatory cycles can really exist in biological regulatory networks, which however have not yet been discovered in real biological systems, or this is only a misleading conclusion due to the errors caused by the discretization of the BM method. In [48,49] the authors proposed a concept of stability analysis of BM cycle solutions, and they found that imposing stability conditions could greatly reduce the number of cycle attractors of BM. It is thus necessary to revisit the same problem by using more accurate and physically meaningful rule, ordinary differential equations.

In this Letter we exhaustively enumerate all the 3-gene networks by using both the ODE and BM methods. In comparison we find that both methods provide practically identical results in most of cases of steady solutions. These identities verify that the simplified BM rule can correctly describe some essences of TR circuits. However, to our great surprise, most of network structures showing periodic cycles with the BM method possess only stationary states in ODE description. The reason is that time and variable discretizations in BM method can introduce oscillations and other complicated types of motions which do not exist in original continuous systems. We extend the investigations to 4-gene, 5-gene and more-gene, and get qualitatively the same results.

2. Model and methods

We adopt a set of ODEs widely used for describing genetic cis-regulation. The ODEs considered AND gate between all repressive regulations and between repressive and active regulations, and OR gate between active regulations. This set of equations were used by some previous biological works [27–29,32,50,51]:

$$\frac{dx_i}{dt} = \mu - \gamma x_i + (1-\mu) f_i(\mathbf{x}),$$

$$f_i = \begin{cases} A_i(\mathbf{x}) & \text{Active regulation,} \\ R_i(\mathbf{x}) & \text{Repressive regulation,} \\ A_i(\mathbf{x}) R_i(\mathbf{x}) & \text{Joint regulation,} \end{cases}$$

$$(\mathbf{x}) = (x_1, x_2, \ldots, x_N),$$

$$A_i(\mathbf{x}) = \frac{act_i^h}{act_i^h + K^h}, \qquad R_i(\mathbf{x}) = \frac{K^h}{rep_i^h + K^h},$$

$$act_i = \sum_{j=1}^{n_{a_i}} x_j, \qquad rep_i = \sum_{j'=1}^{n_{r_i}} x_{j'} \quad (i, j = 1, \ldots, N), \tag{1}$$

where x_i represents the concentration of the ith protein; act_i and rep_i are the sums of concentrations of all the n_{a_i} activators and n_{r_i} repressors, respectively, applied to the ith protein. There are four parameters in Eq. (1). μ is the leakage transcription rate in the absence of activators and the presence of repressors; K is the concentration of activator (repressor) for defining the regulation half-maximal; h stands for the Hill coefficient and γ for the decay rate. In order to compare fairly with the BM method we ignore the intrinsic differences between the different genes and set the parameters homogeneous for the dynamics of all proteins.

A Boolean map discretizing the dynamics of Eq. (1) is also investigated in our work. It can be formalized by the following maps

$$s_i^{t+1} = \begin{cases} 1 \sum_{j=1}^N c_{ij} s_j^t > 0, \\ 1 \sum_{j=1}^N c_{ij} s_j^t = 0 \quad \text{for } n_{a_i} = 0 \text{ and } n_{r_i} > 0, \\ 0 \sum_{j=1}^N c_{ij} s_j^t = 0 \quad \text{for } n_{a_i} > 0 \text{ or } n_{a_i} = n_{r_i} = 0, \\ 0 \sum_{j=1}^N c_{ij} s_j^t < 0, \\ (i = 1, \ldots, N), \end{cases} \tag{2}$$

where s_i^{t+1} is the value of the ith gene at time $t+1$, which depends on the values of all the connected genes s_j^t at time t. $\{c_{ij}\}$ is the connectivity matrix of the network. According to different rules, c_{ij} can adopt diverse values. If we use strong inhibition rule, c_{ij} can be 1, $-\infty$, 0, corresponding to activation, repression and no regulatory reactions, respectively [52]; If we use voting rule [47], c_{ij} can be 1, -1, 0, corresponding to the same cases. In these rules, the time scales of decay and regulation are equal to each other.

We compute the dynamics of both ODEs Eq. (1) and BMs Eq. (2) on few-gene cis-regulatory networks. Auto-regulations are permitted, while external stimuli are excluded (we consider autonomous network dynamics). We exhaustively enumerate all the 3-node networks topologically nonequivalent. Networks that contain isolated nodes are also ruled out from our sets. Totally we investigate 3284 non-isomorphic ally connected 3-node configurations. Our statistics are based on these 3284 configurations unless specified otherwise. We extend the investigations also to some other n-node systems with $n > 3$ ($n = 4$, 5, and more). For larger n's, instead of exhaustive enumeration we randomly choose certain numbers of networks and compare the outputs of these systems with the both methods.

3. Numerical results

3.1. Fixing the control parameters of ODEs for comparison

While the dynamics of BMs depends only on initial conditions, the evolutions of ODEs Eq. (1) depend on both initial conditions and the four control parameters μ, K, h, γ. Therefore we should firstly fix these ODE control parameters for the comparison. We adopt the principle of maximum similarity. Numerically, for 3-gene we pick up 100 networks randomly in all the 3284 networks, set $\gamma = 0.1$ (the reason for the choice of this γ value will be discussed in the end of this section) in Eq. (1), which means that the range of the ODE solutions is between 0 and 10, and then change the other three parameters in Eq. (1). For different parameter sets, we get solutions of ODEs on all 100 networks, and coarse-grain the continuous variables of the asymptotic solutions into the binary values if the solutions are stationary. Namely, if a solution is greater than 5 we value it by 1, and 0 if the solution is less than 5. At the same time, we evolve the strong inhibition BM rule for the same networks. Then we compare the corresponding results by the two methods for eight different initial conditions of all 100 random networks to check whether the two rules provide the same binary solutions, and count for all parameter sets

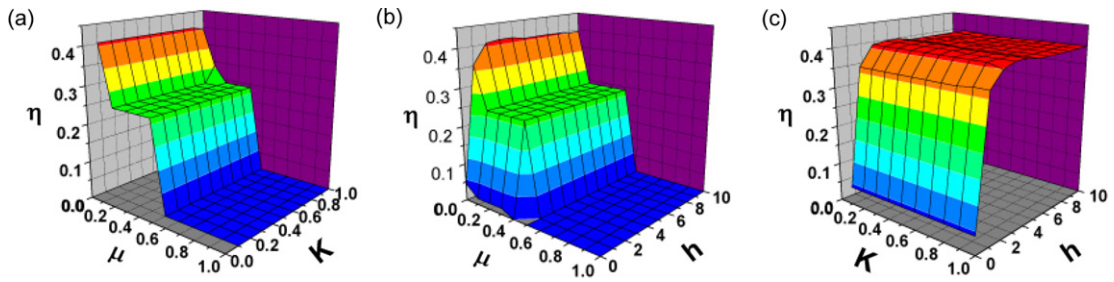

Fig. 1. Identity rate R plotted in μ–K, μ–h and K–h planes, respectively. By 'identity rate' we mean the rate of the number of tests providing exactly the same solutions in ODE and BM rules over the total number of tests (100 networks in our case) randomly chosen for comparison. Parameter range: $\mu \in [0, 1]$, $K \in (0, 1]$, and $h \in [0, 10]$.

tested the percentages of the tests with which the two methods provide completely identical attractors. Here with the BM rule we define an "attractor" by any asymptotic state, while with ODE we define an attractor by an asymptotic state attracting the trajectories initially in its vicinity. Here pure zero solutions (i.e., in those solutions all genes take zero protein concentration) are excluded from our statistics because this state shows no biological activity and is thus regarded to be trivial. In Fig. 1(a)–(c) we plot the identity rates in μ–K, μ–h and K–h planes, respectively. It is found that small μ and relatively large h favor to high identity rates. In particular, we observe that the parameter set ($\mu = 0.0$, $K \approx 0.3$, $h \approx 2.0$) yields the largest identity percentage $\eta \approx 41\%$. We then repeat the comparisons for another 100-networks randomly chosen (without overlap with the first 100-networks), we got a new set of maximum identity parameters (μ, K, h) and find that this set is practically the same as the previous set. Therefore, the parameter set ($\mu = 0.0$, $K = 0.3$, $h = 2.0$) will be used in the following for performing the systematical comparison of the two methods. We find that similar parameter values have been used in many previous investigations of practical biological TRs [24,27–29,32].

Finally, we choose the $\gamma = 0.1$ for two reasons. On the one hand, for stationary solutions the comparisons of ODE and BM are insensitive to the change of γ. On the other hand, periodic solutions of ODEs are rather sensitive to γ. with $\gamma = 1$ ODEs have almost no periodic solution. The number of periodic solutions of ODE increases as γ decreases, and this number saturates for sufficiently small γ. Since BMs have atypically many periodic solutions we choose $\gamma = 0.1$ for favoring the consistence between ODE and BM for periodic solutions.

3.2. Satisfactory agreement between ODE and BM rules in describing stationary attractors

In Tables 1 and 2 we make a systematical comparison between the ODE and BM methods by exhaustively enumerating all 3-gene networks. In Table 1, all items have the following meanings:

"TN" ("TP") represents the numbers of tests which have nonzero steady solutions (periodic solutions) for the given regulatory structures and initial conditions.

"OTN" ("OTP") represents the numbers of all tests from same initial conditions with which both ODE and BM rules have steady (periodic) solutions.

"IOTN" represents the number of all tests from same initial conditions with which both ODE and BM have identical steady solutions.

"RTN" is the proportion of IOTN to OTN, i.e., $RTN = IOTN/OTN$.

For asymptotic periodic states of ODE systems, we considered only the fact whether the both ODE and BM systems produce periodic states from the same initial conditions (OTP), while we did not specify whether the two algorithms yield "identical periodic orbits".

Table 1
A systematical comparison of numerical results between the BM and ODE descriptions. We scan all the 8 initial conditions in all 3284 networks with BM and ODE description. The tests producing pure zero solution are excluded. The meanings of all items are explained in the text. In this table we observe that both the ODE and BM methods coincide satisfactorily in describing stationary states while deviate considerable in predicting periodic orbits.

Rules	Tests	
	Nonperd	Perd
BM	8485	13263
ODE	16983	55
Overlap	8391 (OTN)	22 (OTP)
IOTN	7304	
RTN	87.05%	

Table 2
A frequency statistics of networks and tests with various steady solution attractors. We count the number of steady networks in the total 3284 networks that contain the same multiple steady attractors (all pure-zero solution attractors are excluded from our counting). "OF (OTF)" means the numbers of networks (the number of all tests from these networks) producing the same numbers of attractors by using both BM and ODE rule descriptions. "IF (ITF)" means the numbers of networks (tests) among OF (OTF) possess exactly the same steady attractors with both rules. "RF (RTF)" is the proportions of IF (ITF) to OF (OTF).

Rules\No. att	1	2	3	4	5	Total
BM	648	299	76	16	0	1039
ODE	1800	550	100	18	1	2469
OF	628	244	65	16	0	953
IF	459	165	41	11	0	676
RIF	73.09%	67.62%	63.08%	68.75%	0	70.93%
OTF	4020	1675	446	108	0	6249
ITF	3483	1521	417	104	0	5525
RTF	86.64%	90.81%	93.50%	96.30%	0	88.41%

We find that almost all of the 3-gene networks have stationary solutions with the ODE rule (the ratio of stable steady solution is as high as 99.44%). However, with the BM rule, this ratio is lowed to 34.2%. Note, in computing these ratios we exclude all pure-zero solutions. From the view of IOTN, the identical rate (IOTN) is very high. On the other hand, the absolute majority of periodic motions revealed by BMs are absent in ODE.

In Table 2 we pick up all the networks which can evolve into stationary states from all initial conditions with ODE or BM dynamics, and then classify the networks by numbers of attractors. Here again all pure-zero solutions are excluded from our statistics. The items in Table 2 have the following meanings.

"OF" means the numbers of networks producing the same numbers of attractors by using both BM and ODE rule descriptions.

"IF" represents all the networks among OF which have identical steady solutions from all initial conditions with both ODE and BM.

"RIF" is the proportion of IF to OF, i.e., $RIF = IF/OF$.

"OTF" stands for the numbers of tests (among the OF) having nonzero steady solutions with both ODE and BM from same initial conditions.

"ITF" represents all the tests among OTF which possess exactly the same solutions with both rules.

"RITF" is the proportion of ITF to OTF, i.e., $RITF = ITF/OTF$.

From the rates, we find again that the predictions of the BM rule represent the results of the more accurate ODE description very well in the cases stationary asymptotic states. Therefore we expect that both ODE and BM methods widely used in the analysis of biological TR networks can well and equivalently (in the sense of binary discretization) describe the behaviors of these TR systems for stationary states.

3.3. Sharp deviation between ODE and BM rules in predicting periodic oscillations

However, when periodic cycles are considered, the results of ODE and BM rules become sharply different. With the BM rules Nochomovitz et al. found atypically many networks providing periodic oscillatory states. By exhaustive searching in the 3284 3-gene networks we find 2001 networks having totally 2263 periodic attractors (some networks have more than one oscillatory attractors). To our great surprise that by exhaustive searching in the 3284 3-node networks with the ODE rule and homogeneous parameters we find only 14 networks having totally 14 periodic attractors (each such network has only one oscillatory attractor). Then the characteristic feature of atypically many periodic cycles predicted by the BM method are not observed in the corresponding more accurate ODE systems, and the mechanisms underlying these serious and essential deviations should be investigated.

3.4. Comparison of ODE and BM in n-node circuits with $n > 3$ and seldomness of periodic cycles and extreme rareness of chaotic motions in TR circuits

In order to confirm the general applicability of the above conclusions, we also study n-gene circuits with $n > 3$. We first use the method of Fig. 1 to fix the parameter values of Eq. (1) for the best matching between the ODE (Eq. (1)) and BM (Eq. (2)) methods, and find that the parameter set used in Tables 1 and 2 are also suitable for $n > 3$.

With $n > 3$ the number of topologically distinctive networks become huge. For $n = 4$, 5 and 10, instead of exhaustive searching for each n we make large numbers of tests for 4-node, 5-node and 10-node with different topologically distinctive networks. The networks are such randomly chosen that any given node A can receive from any node B (B may be another node or node A itself) a positive or negative interaction, or no interaction all with 1/3 probability. We show in Table 3 the results of such 5000 4-node, 5000 5-node, and 1000 10-node tests having only stationary states with BMs Eq. (2), while show in Table 4 the other results of same numbers of tests having at least one periodic cycles with BM dynamics. We then numerically compute the ODEs Eq. (1) for all these networks and compare the asymptotic solutions of ODEs (in binary discretization) with those obtained by the BM rule. The comparisons are given in Tables 3 and 4. In all cases of 4, 5 and 10 nodes we observe: when BMs contain only steady solutions the overlap and identical rates between the two methods are high (about 88.17% for 4-node networks, 90.18% for 5-node networks and 84.84% for 10-node networks). Even in cases of that BMs show periodic motions with certain initial conditions (Table 4), ODE and BM have still rather large rates for the identical steady attractors. All these observations are similar to those of 3-node systems.

From Tables 1 and 4, it is clear that for most of periodically oscillatory BM networks the ODE method show only stable stationary solutions. Another interesting observation for $n \geqslant 3$ is extreme seldomness of chaotic states. It is well known that nonlinear ODEs with dimension $n \geqslant 3$ may easily show chaotic motions. However, we found surprisingly that chaotic attractors are extremely seldom in such typically nonlinear and high-dimensional TR systems. Specifically, we have tested a huge number of TR circuits for $n \geqslant 3$ in wide biologically accepted regions, with both homogeneous and inhomogeneous parameter distributions. We found no chaos in over million of tests for $n = 3$, 4, and found three tests of chaos among 2×10^5 tests for $n > 5$. In Refs. [53–55] authors studied chaotic behaviors of TR circuits. In particular, chaotic motion was observed for TR circuits with piecewise linear dynamics. More detailed investigation on chaotic behavior will be our future works.

Nevertheless, from the above investigations we can conclude that with the ODE rule periodic and chaotic oscillations are not frequent in autonomous biological regulatory circuits. This conclusion is sharply different from the results revealed by the BM rule which explores atypically many periodic motions [47]. However, it agrees with the biological observations that biological circuits often possess stable stationary states. Periodic and chaotic oscillations in few-node autonomous circuits have been found very seldom [24,56], and most of oscillatory cycles in TR networks are caused by external periodic stimuli [32,43,57,58].

Table 3
A statistics in 5000 randomly-chosen 4-node, 5-node and 1000 randomly-chosen 10-node networks which possess only steady attractors with the BM rule. For each network we test all 16 sets of initial conditions for $n = 4$ and 16 different sets of initial conditions randomly chosen for $n = 5$, 10. All the parameter values and the meanings of all items are the same as those in Table 1.

Node	Rules	Tests	
		Nonperd	Perd
4	BM	65803	0
	ODE	70915	269
	Overlap	64220 (OTN)	0 (OTP)
	IOTN	56624	
	RTN	88.17%	
5	BM	65478	0
	ODE	70910	435
	Overlap	63481 (OTN)	0 (OTP)
	IOTN	57244	
	RTN	90.18%	
10	BM	11502	0
	ODE	13590	164
	Overlap	10950 (OTN)	0 (OTP)
	IOTN	9290	
	RTN	84.84%	

Table 4
A statistics in 5000 randomly-chosen 4-node, 5-node and 1000 randomly-chosen 10-node networks which possess at least one periodic attractors with the BM rule. The same as Table 3 with networks having at least one periodic attractors with the BM rule. All items have the same meanings as Table 1.

Node	Rules	Tests	
		Nonperd	Perd
4	BM	8289	64945
	ODE	52881	437
	Overlap	8119 (OTN)	274 (OTP)
	IOTN	7327	
	RTN	90.25%	
5	BM	8346	63405
	ODE	56597	557
	Overlap	8163 (OTN)	407 (OTP)
	IOTN	7252	
	RTN	88.84%	
10	BM	1018	11362
	ODE	11627	168
	Overlap	959 (OTN)	104 (OTP)
	IOTN	803	
	RTN	83.73%	

4. Mechanism underlying the differences between ODE and BM rules

In the field of nonlinear dynamics it has been known that various discretizations may cause some complex dynamics such as oscillations and chaos which do not exist in the original continuous systems. And these false oscillations are found indeed in our case when the ODE dynamics is transformed to the BM dynamics by the variable and time discretizations. It has still not been well understood why in n-node nonlinear regulatory ODEs sustained oscillations are so rare. Here we can only demonstrate intuitively the differences between the ODE and BM rules when periodic oscillations are involved. In Figs. 2 and 3 we compare the evolutions of two 3-gene TR circuits with both ODE and BM dynamics. In both networks initial conditions are chosen in one of the Boolean states. From the networks we observe that all the tendencies of ODE and BM dynamics are identical when the trajectories start from the initial states. In case of stable steady solutions these identical tendencies result in identical asymptotic states of the two rules (Fig. 2(b)). However, when the BM rule shows periodic solutions, a basin of steady solution of ODE (black square) is in the middle-way of the oscillation path of the BM state. By the approximation of variables and time discretizations the BM rule simply jumps over this middle-way attracting basin, and replace the steady state of ODE by periodic oscillation of BM $R \to S \to P \to R$ as shown in Fig. 3(b).

Now it is interesting to compare our exhaustive numerical results with some previous theoretical and numerical analysis. Let us take 3-node tests as examples. First, we found that for most of BM steady states we obtain discretizely equivalent ODE states with identical initial conditions. These observations support the conclusion in [38,44,45] about regular steady points (RSPs). We guess that some very small portions of deviations of RSPs may be due to the fixed control parameters of ODEs. Second, for all $N_p = 13263$ BM periodic tests we studied the exhaustively stability of the orbits by applying the method suggested in [48], we found that the orbits of 6199 tests are unstable. These observations are consistent with the conclusion that less than half of cycles of BMs may be not actual attractors of the circuits. By applying the method suggested in [49], we found further that more than 3500 tests periodic states of BMs could lead to stationary states with asynchronous update algorithms, among which about 1500 these tests of new stationary solutions are identical steady solution of ODEs from same initial values. Finally, we took few examples, when BMs show periodic orbits, to study the possible singular steady point (SSP) of BMs, and we found that in some of these examples the BM systems contain SSPs which are identical to the corresponding steady attractive states of ODEs. In particular, in case of Fig. 3, the periodic orbit of BM circuit is unstable, and then the BM system has indeed a SSP which is the same as the steady state of the corresponding ODE shown in Fig. 3(b).

However, there are also a few converse results. The BM rule shows steady solutions while the ODE rule shows periodic solutions. In Fig. 4, we demonstrate this case. All of the initial states evolve into the same steady states $O(0,0,0)$, $S(0,0,1)$ or $P(1,0,0)$ by both ODE and BM rules, except the initial value $N(1,1,1)$ and $Q(1,1,0)$. By the BM rule N evolves into state O, and Q evolves into state S. However, with the ODE rule the system evolves into a periodic solution instead of the pure zero state O because there is an unstable focus near this O state (with the variable discretization the BM rule does not feel the difference between stable and unstable focuses).

It is interesting to extend our investigation to the general problem of oscillatory behaviors of the cis-regulation of Eq. (1). We had made exhaustive investigations of 3-node ODE systems in all 3284 networks with different homogeneous control parame-

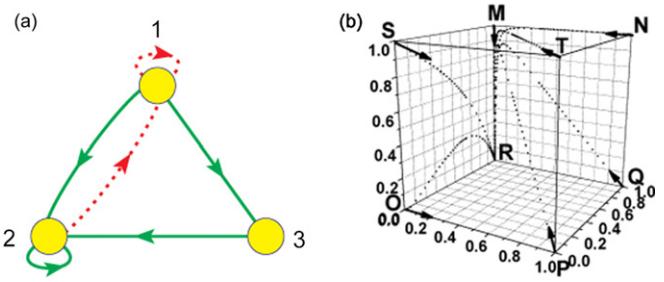

Fig. 2. Intuitive illustration on ODE and BM dynamics possessing exactly the same attractors. (a) The diagram of the transcriptional regulatory circuit for the comparison. The green solid lines present activation and red dotted lines for repression. Arrows indicate the directions of regulations. (b) Trajectories of ODE and BM from eight different initial conditions shown in the phase space. The dotted line is the orbits of ODE dynamics, and the arrows mark the directions of BM dynamics at the starting points. We can see that trajectories of ODE and BM from all initial conditions approach the same steady state $R(0,1,0)$ practically along the tangent directions indicated by arrows. For BMs: $O \to P \to M \to R \to R$; $S \to Q \to M \to R \to R$; $N \to M \to R \to R$; $M \to R \to R$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this Letter.)

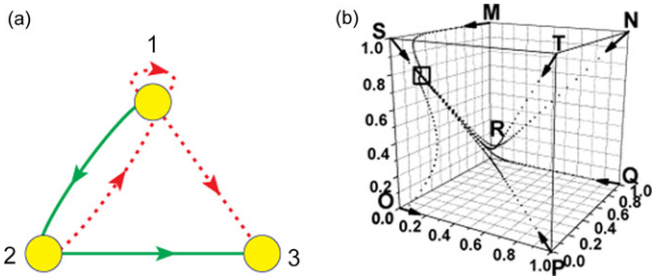

Fig. 3. The same as Fig. 2 with another network investigated to intuitively illustrate why a ODE circuit possesses a steady state while the BM dynamics shows a periodic cycle. (a) The diagram of the transcriptional regulatory circuit for the comparison. (b) The BM trajectories evolve to a periodic asymptotic orbit $R \to S \to P \to R \to \cdots$ as: $O \to P \to R \to S \to P \to R \to \cdots$; $M \to S \to P \to R \to S \to P \to R \to \cdots$; $T \to R \to S \to P \to R \to \cdots$; $Q \to R \to S \to P \to R \to \cdots$; $N \to R \to S \to P \to R \to \cdots$. The ODE trajectories asymptotically approach a steady state $S(0,0,1)$ in binary representation. Note, the tendencies of ODE and Boolean dynamics are the same in their common initial conditions. However, the orbits of ODE go asymptotically to the attractor denoted by the black square due to some basins of attraction in the middle way. In contrast, with the BM dynamics all the trajectories evolve to a periodic orbit $R \to S \to P \to R$ which just jumps over the steady state of the ODE system due to time and variable discretizations.

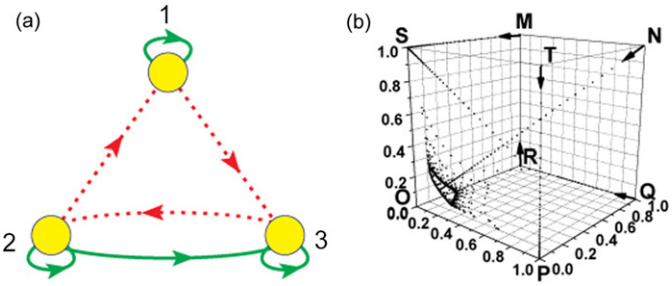

Fig. 4. The same as Fig. 2 with a third network investigated to intuitively illustrate why an ODE circuit shows a periodic cycle while the BM dynamics possesses a steady state. (a) The diagram of the transcriptional regulatory circuit for the comparison. (b) The BM trajectories approach the steady state $O(0,0,0)$, $S(0,0,1)$ or $P(1,0,0)$ from different initial values. $T \to P$; $N \to O$; $Q \to R \to M \to S$. In contrast, with the ODE dynamics, two of the trajectories approach the same steady state $S(0,0,1)$, one of the trajectories approaches the steady state $P(1,0,0)$, but the initial value $N(1,1,1)$ and $Q(1,1,0)$ evolves to a periodic cycle. By the linear stability analysis, there is an unstable focus near $O(0,0,0)$, and this is the reason (instability of the steady solution) why generic initial conditions will robustly evolve into periodic states by ODE rule while BM does not feel the difference of stable or unstable focuses and shows steady state.

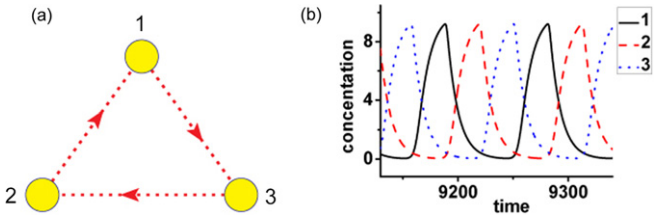

Fig. 5. Periodic motion of a circuit of three genes with successive repressing actions. (a) The diagram of a regulatory 3-gene successive repressive circuit that has periodic oscillatory solution by using ODE rule. (b) The asymptotic periodic evolution of the circuit A at $\mu = 0.0$, $K = 0.3$, $h = 5.0$, $\gamma = 0.1$.

ters randomly chosen (uniform probability density in the ranges $\mu \in [0.0, 1.0]$, $K \in [0.1, 1.0]$, and $h \in [1, 10]$). Totally, we performed tests of 3284 networks $\times$ 10 parameter sets $\times$ 8 initial conditions, and found no any chaos and found less than 0.5% tests producing oscillatory cycles, one of which is a circuit of three proteins with successive repressing interactions shown in Fig. 5(a). And the corresponding asymptotic oscillatory evolutions of the three nodes are presented in Fig. 5(b). The circuit Fig. 5(a) was experimentally realized in [24] where biological oscillation of the 3-node autonomous circuit was observed indeed for the first time. We have also tested a large number of n-node systems for $n = 4$, 5, 10, 20 and other larger n also with homogeneous control parameters randomly chosen. We found that the cases of periodic oscillation increases as n increases, but the ratios of the numbers of oscillatory solutions over the total tests are still very low.

5. Discussion

Ordinary differential equations and Boolean maps are two major approaches currently used for describing gene transcriptional regulatory circuits. In the present work we have made systematical comparison between the two descriptions by considering few-node autonomous networks. By exhaustive enumerate of 3-node systems and extensive tests of n-node systems with $n > 3$ we find that the two descriptions agree satisfactorily with each other for exploring asymptotic steady states. On the other hand, these two descriptions deviate from each other considerably for predicting periodic oscillatory cycles. We also make a systematical comparison between the ODE rule and BM with voting rule [47,59], and we get the similar result.

While abundant n-node ($n \geqslant 3$) networks show periodic oscillations with the BM rule the corresponding ODE systems show oscillatory cycles much rarely. We explain that in these cases BMs give wrong predictions due to the approximations of variable and time discretizations. Though it is well known in nonlinear dynamics that various discretizations may induce complex motions including oscillations which do not exist in the original continuous systems, the above observations are still greatly surprising. We are familiar with that oscillations and chaos exist widely in nonlinear dynamical systems when the dimension of the variable space is sufficiently large. In our case we expect that oscillations and chaos should be very easily observed in n-gene TR circuits (periodic oscillations for $n \geqslant 2$ and chaos for $n \geqslant 3$). For all the systems tested in this Letter, the BM method shows atypically many oscillatory motions. It is therefore really strange that with our extensive tests of ODE circuits we have found very small fraction of tests showing oscillations. Nevertheless, these features are very important and greatly welcome in biological systems for the stability and controllability of the functions of biological networks: autonomous TR circuits can be prepared ready in their stable steady states, waiting for the external stimuli to perform various ordered biological rhythm, including oscillatory cycles.

Acknowledgements

This work was supported by the National Natural Science Foundation of China under Grant No. 10975015 and by the National Basic Research Program of China (973 Program) under Grant No. 2007CB814800.

References

[1] S. Kauffman, J. Theor. Biol. 22 (1969) 437.
[2] L. Glass, K. Sa, J. Theor. Biol. 39 (1973) 103.
[3] J. Doebley, L. Lukens, Plant Cell 10 (1998) 1075.
[4] S. Tavazoie, J.D. Hughes, M.J. Campbell, R.J. Cho, G.M. Church, Nat. Genet. 22 (1999) 281.
[5] P.X. Xu, X. Zhang, A. Heaney, S. Yoon, A.M. Michelson, R.L. Maas, Development 126 (1999) 383.
[6] K.J. Peterson, E.H. Davidson, Proc. Natl. Acad. Sci. USA 97 (2000) 4430.
[7] G. von Dassow, E. Meir, E. Munro, M. Garett, Nature 406 (2000) 188.
[8] T.I. Lee, N.J. Rinaldi, F. Robert, et al., Science 298 (2002) 799.
[9] J. Ihmels, G. Friedlander, S. Bergmann, O. Sarig, Y. Ziv, N. Barkai, Nat. Genet. 31 (2002) 370.
[10] H.W. Ma, B. Kumar, U. Ditges, F. Gunzer, J. Buer, A.P. Zeng, Nucleic Acids Res. 32 (2004) 6643.
[11] H.H. McAdams, B. Srinivasan, A.P. Arkin, Nature Rev. Genet. 5 (2004) 1.
[12] W. Ma, L. Lai, Q. Ouyang, C. Tang, Mol. Syst. Biol. 2 (2006) 70.
[13] G. Posfai, G.E.A. Plunkett III, Science 312 (2006) 1044.
[14] M. Gilchrist, V. Thorsson, B. Li, A.G. Rust, M. Korb, K. Kennedy, T. Hai, H. Bolouri, A. Aderem, Nature 441 (2006) 173.
[15] C.G. Kurland, L.J. Collins, D. Penny, Science 312 (2006) 1011.
[16] L. Mendoza, I. Xenarios, Theoretical Biology and Medical Modelling 3 (2006) 13.
[17] E. Remy, P. Ruet, D. Thieffry, Adv. Appl. Math. 41 (2008) 335.
[18] D. Wittmann, J. Krumsiek, J. Saez-Rodriguea, D. Lauffenburger, BMC Syst. Biol. 3 (2009) 98.
[19] D. Wittmann, F. Blochl, D.E.A. Trumbach, PLoS Comput. Biol. 5 (2009) e1000569.
[20] S.S. Shen-Orr, R. Milo, S. Mangan, U. Alon, Nat. Genet. 31 (2002) 64.
[21] R. Milo, S. Shen-Orr, S. Itzkovitz, N. Kashtan, D. Chklovskii, U. Alon, Science 298 (2002) 824.
[22] A.P. Arkin, Curr. Opin. Biotechnol. 12 (2001) 638.
[23] Y. Yokobayashi, R. Weiss, F.H. Arnold, Proc. Natl. Acad. Sci. USA 99 (2002) 16587.
[24] M.B. Elowitz, S. Leibler, Nature 403 (2000) 335.
[25] C.C. Guet, M.B. Elowitz, W. Hsing, S. Leibler, Science 296 (2002) 1466.
[26] L.F. Wu, T.R. Hughes, A.P. Davierwala, M.D. Robinson, R. Stoughton, S.J. Altschuler, Nat. Genet. 31 (2002) 255.
[27] S. Mangan, U. Alon, Proc. Natl. Acad. Sci. USA 100 (2003) 11980.
[28] S. Mangan, A. Zaslaver, U. Alon, J. Mol. Biol. 334 (2003) 197.
[29] N. Rosenfeld, U. Alon, J. Mol. Biol. 329 (2003) 645.
[30] D.M. Wolf, A.P. Arkin, Curr. Opin. Microbiol. 6 (2003) 125.
[31] E. Yeger-Lotem, S. Sattath, N. Kashtan, S. Itzkovitz, R. Milo, R.Y. Pinter, U. Alon, H. Margalit, Proc. Natl. Acad. Sci. USA 101 (2004) 5934.
[32] S. Ishihara, K. Fujimoto, T. Shibata, Genes to Cells 10 (2005) 1025.
[33] J. Tyson, H.G. Othmer, Prog. Theor. Biol. 5 (1978) 2.
[34] R.D. Bliss, P.R. Painter, A.G. Marr, J. Theor. Biol. 97 (1982) 177.
[35] H. Smith, J. Math. Biol. 25 (1987) 169.
[36] I. Nachman, A. Regev, N. Friedman, Bioinformatics 20 (2004) i248.
[37] E. Dekel, S. Mangan, U. Alon, Phys. Biol. 2 (2005) 81.
[38] S.H. Ei, R. Thomas, Bulletin of Mathematical Biology 55 (1993) 973.
[39] R. Somogyi, C. Sniegoski, Complexity 1 (1996) 45.
[40] J. Boden, J. Theor. Biol. 188 (1997) 391.
[41] M.B. Eisen, P.T. Spellman, P.O. Brown, D. Botstein, Proc. Natl. Acad. Sci. USA 95 (1998) 14863.
[42] X.L. Wen, S. Fuhrman, G. Michaels, D. Carr, S. Smith, J. Barker, R. Somogyi, Proc. Natl. Acad. Sci. USA 95 (1998) 334.
[43] F. Li, T. Long, Y. Lu, Q. Ouyang, C. Tang, Proc. Natl. Acad. Sci. USA 101 (2004) 4781.
[44] T. Mestl, E. Plahte, S.W. Omholt, J. Theor. Biol. 176 (1995) 291.
[45] E. Plahte, T. Mestl, S.W. Omholt, J. Math. Biol. 36 (1998) 321.
[46] J.H. de, J. Gouze, C. Hernandez, M. Page, T. Sari, Bull. Math. Biol. 66 (2004) 301.
[47] Y.D. Nochomovitz, H. Li, Proc. Natl. Acad. Sci. USA 103 (2006) 4180.
[48] K. Klemm, S. Bornholdt, Physical Review E 72 (2005) 55101.
[49] M. Chavez, R. Albert, E. Sontag, J. Theor. Biol. 235 (2005) 431.
[50] W.-M. Ye, X.-D. Huang, X.-H. Huang, P.-F. Li, Q.-Z. Xia, G. Hu, Physics Letters A 374 (2010) 2521.

[51] C. Li, L. Chen, K. Aihara, IEEE 53 (2006) 2451, doi:10.1109/TCSI.2006.883882.
[52] Y. Wu, X. Zhang, J. Yu, Q. Ouyang, PLoS Computational Biology 5 (2009) e1000442, doi:10.1371/journal.pcbi.1000442.
[53] T. Mestl, C. Lemay, L. Glass, Physica D: Nonlinear Phenomena 98 (1996) 33.
[54] R. Bagley, L. Glass, J. Theor. Biol. 183 (1996) 269.
[55] T. Mestl, R. Bagley, L. Glass, Phys. Rev. Lett. 79 (1997) 653.
[56] J.R. Pomerening, S.Y. Kim, J.E. Ferrell, Cell 122 (2005) 565.
[57] N. Rosenfeld, J.W. Young, U. Alon, P.S. Swain, M.B. Elowitz, Science 307 (2005) 1962.
[58] A. Takamatsu, R. Tanaka, T. Fujii, Phys. Rev. Lett. 92 (2004) 228102.
[59] T.Y.-C. Tsai, Y.S. Choi, W. Ma, J.R. Pomerening, T. Chao, J.E. Ferrell, Science 321 (2008) 126.