Commun. Theor. Phys. (Beijing, China) **49** (2008) pp. 1639–1642

Vol. 49, No. 6, June 15, 2008

Nonchaoticity of Ordinary Differential Equations Describing Autonomous Transcriptional Regulatory Circuits*

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(Received March 20, 2008)

Abstract *Gene transcriptional regulation (TR) processes are often described by coupled nonlinear ordinary differential equations (ODEs). When the dimension of TR circuits is high (e.g. $n \geq 3$) the motions of the corresponding ODEs may, very probably, show self-sustained oscillations and chaos. On the other hand, chaoticity may be harmful for the normal biological functions of TR processes. In this letter we numerically study the dynamics of 3-gene TR ODEs in great detail, and investigate many 4-, 5-, and 10-gene TR systems by randomly choosing figures and parameters in the conventionally accepted ranges. And we find that oscillations are very seldom and no chaotic motion is observed, even if the dimension of systems is sufficiently high ($n \geq 3$). It is argued that the observation of nonchaoticity of these ODEs agrees with normal functions of actual TR processes.*

PACS numbers: 87.10.Vg
Key words: transcriptional regulatory circuit, periodic oscillation, nonchaoticity

In the recent decade the problem of gene expressions in biological regulatory transcriptional networks has attracted great attention of biologists, and other scientists working in crossing fields.[1−7] Mathematically, for exploring the principle and relationship of transcriptional regulations (TR) two major methods have been widely used: ordinary differential equations (ODE) and Boolean maps (BM). The former is regarded to be a more accurate description of TR processes while the latter is certainly approximation of the former with variable and time discretizations. For ODE description, TR processes can be studied in the following equations coupled in various regulatory networks (after certain approximations)[8−10]

$$\begin{aligned}
&\frac{\mathrm{d}p_i}{\mathrm{d}t} = \mu_i + (1-\mu_i)\frac{\mathrm{act}_i^{\nu_i}}{\mathrm{act}_i^{\nu_i}+\kappa_i^{\nu_i}}\frac{\kappa_i^{\nu_i}}{\mathrm{rep}_i^{\nu_i}+\kappa_i^{\nu_i}} - \gamma_i p_i\,,\\
&\mathrm{act}_i = \sum_j \alpha_j^{(i)} p_j\,, \quad \mathrm{rep}_i = \sum_j \beta_j^{(i)} p_j\,,\\
&\qquad (i,j=1,\ldots,n)\,. \qquad (1)
\end{aligned}$$

If there is no activation to a gene, the equations can be simplified to the following form

$$\begin{aligned}
&\frac{\mathrm{d}p_i}{\mathrm{d}t} = \mu_i + (1-\mu_i)\frac{\kappa_i^{\nu_i}}{\mathrm{rep}_i^{\nu_i}+\kappa_i^{\nu_i}} - \gamma_i p_i\,,\\
&\qquad (i,j=1,\ldots,n)\,. \qquad (2)
\end{aligned}$$

In Eqs. (1) and (2), p_i represents the concentration of the i-th protein; act_i and rep_i are the sum of concentrations of all the activators and the sum of the concentrations of all the repressors applied to the i-th protein, respectively. $\alpha_j^{(i)}$ ($\beta_j^{(i)}$) is the weight of the concentration of the j-th protein activating (repressing) the expression of the i-th gene. There are four parameters in Eq. (1) besides $\alpha_j^{(i)}$ and $\beta_j^{(i)}$. For the equation of the i-th protein μ_i is the basal transcription rate in the absence of activators and repressors; κ_i is the concentration of activator (repressor) for defining the regulation half-maximal; ν_i stands for the Hill coefficient and γ_i for the decay rate.

In autonomous cases, equation (1) is a typical set of coupled nonlinear ODEs. In the past decades we have been familiar with the complex behaviors of nonlinear systems. It is generally expected that nonlinear ODEs, like Eq. (1), may probably show self-sustained oscillations for $n \geq 2$, chaotic motions for $n \geq 3$ and quasiperiodic motions for $n \geq 4$. However, in actual biological TR circuits, investigations performed so far showed that most of autonomous TR systems (for $n \geq 3$) have stationary asymptotic states, and oscillations are usually induced by periodic external stimuli.[5,8,11−13] Moreover, chaos which may badly affect biological TR processes is extremely seldom (Actually, so far no observation of chaos has been reported when realistic biological TR circuits function normally). These observations seem to be contradictory with the general expectation from the nonlinearity and high dimensionality of Eq. (1) (e.g. for $n \geq 3$).

*The project supported by National Natural Science Foundation of China under Grant Nos. 10335010 and 70431002, and the Nonlinear Science 973 Project under Grant No. 10675020

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From the above discussions we expect while oscillations and chaos may be very easily observed in ODEs describing n-gene autonomous TR circuits for $n \geq 3$, actual biological TR processes seem not to support this perspective. It is thus interesting to ask whether the complex behaviors of general nonlinear and high-dimensional ODEs really exist in the particular ODEs (1) for biological TR networks. And it is the central task of the present letter to answer this question.

Let us start our study by a systematical investigation of 3-gene TR circuits.[12,14] For specific, we first consider homogeneous parameter distributions and fix $\mu_i = 0.0$, $\kappa_i = 0.3$, $\nu_i = 2$, $\gamma_i = 1.0$. With these parameters we exhaustively compute Eq. (1) for all irreducible figures: each gene can regulate one, or two, or three (self-regulation is acceptable) proteins with either activative or repressive regulation. Altogether we have examined 3284 irreducible figures which are complete for 3-gene circuits. For each figure we run Eq. (1) from eight different initial conditions, i.e., each protein can take initial value 0 or 1. In Table 1 we show the number of figures having different multiplicities of attractors. An interesting as well as surprising observation is that in all these 5076 attractors, we find only stationary stable solutions. No oscillation and no chaos have been revealed. In Fig. 1 we show time evolutions of two figures arbitrarily chosen (Figs. 1(a) and 1(d)), and we find typical motions from different initial conditions to a single attractor (Fig. 1(b)) and to multiple attractors (Fig. 1(e)).

Table 1 A frequency statistics of figures with different number of attractors. Totally, we observe 5076 attractors by running Eq. (1) with all 3284 figures. Item 'All' represents the number of figures with the given multiplicity of attractors. And item 'Perd' indicates the number of figures in 'All' that produce periodic oscillatory attractors.

Rules\No. att	1		2		3		4		5		6	
	All	Perd	All	Perd	All	Perd	All	Perd	All	Perd	All	Perd
ODE	1810	0	1208	0	219	0	43	0	3	0	1	0

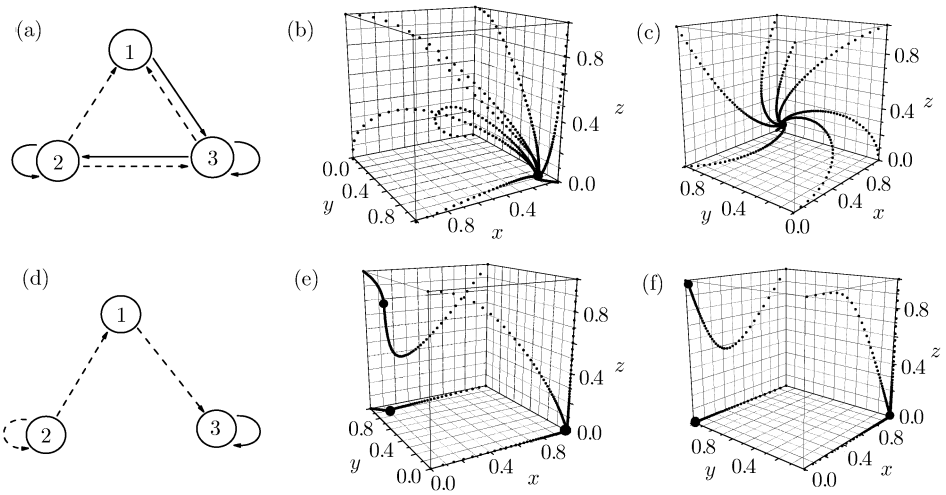

Fig. 1 Typical motions of 3-gene circuits. (a) Diagram of a randomly-chosen 3-gene circuit, which has only one stable node. Solid lines represent activation and dashed lines for repression. Arrows indicate the directions of regulation. (b) Evolution trajectories of system (a) shown in 3-D phase space from eight different initial conditions. Parameters are taken homogeneously as: $\mu_i = 0.0$, $\kappa_i = 0.3$, $\nu_i = 2.0$, $\gamma_i = 1.0$, $\alpha_j^{(i)} = \beta_j^{(i)} = 1.0$, $i, j = 1, 2, 3$. (c) The same as (b) with partially heterogeneous parameters, randomly chosen. $\kappa_1 = 0.8$, $\kappa_2 = 0.1$, $\kappa_3 = 0.4$, $\nu_1 = 1.0$, $\nu_2 = 5.0$, $\nu_3 = 2.0$, $\mu_i = 0.0$, $\gamma_i = 1.0$, $\alpha_j^{(i)} = \beta_j^{(i)} = 1.0$, $i, j = 1, 2, 3$. (d) The same as (a) with another randomly-chosen 3-gene circuit which has three different attractors. (e) and (f) The same as (b) and (c), respectively with (d) investigated. In (f) the actual parameters are $\kappa_1 = 0.4$, $\kappa_2 = 0.1$, $\kappa_3 = 0.8$, $\nu_1 = 5.0$, $\nu_2 = 3.0$, $\nu_3 = 5.0$, $\mu_i = 0.0$, $\gamma_i = 1.0$, $\alpha_j^{(i)} = \beta_j^{(i)} = 1.0$, $i, j = 1, 2, 3$.

In the above discussions all different proteins take the same control parameters. We now go further to consider parameter heterogeneity of proteins. For each figure we randomly take 10 parameter sets in the range $\mu_i = 0.0$, $\kappa_i \in (0.0, 0.5)$, $\nu_i \in [1, 10]$, $\gamma_i \in (0.0, 10.0]$, $\alpha_j^{(i)} \in (0.0, 1.0]$, $\beta_j^{(i)} \in (0.0, 1.0]$. These parameter regions have been widely used in previous works studying TR circuits,[8,15,16] and for each figure and each set of parameters we randomly choose

4 initial conditions, and then we compute Eq. (1) for each figure with each parameter set and each initial condition randomly chosen. In all these 3284×10 figure and parameter tests we observe only 6 tests in 4 figures having periodic cycles, and absolute majority of tests produce asymptotic steady states and no any chaotic motion has been found. Some typical motions of stable states are shown in Figs. 1(c) and 1(f).

For 3-gene TR circuits we have made exhaustive investigation for the dynamics of all regulation figures, and confirmed seldomness of oscillation and nonchaoticity in autonomous 3-gene TR systems. Intuitively, oscillation and chaos have more probability to appear in n-gene circuits as n increases. It is natural to extend the study to n-gene systems with $n > 3$. We also compute Eq. (1) by taking $n = 4, 5$, and 10, respectively, each for 100 figures randomly chosen. For each figure we randomly choose 5 sets of heterogeneous parameters and for each parameter set we start from 4 initial conditions. In all these 6000 runs we again find that most of asymptotic solutions (more than 99% tests) are stationary. Less than 1% tests reveal periodic oscillations and none of the tests shows chaoticity.

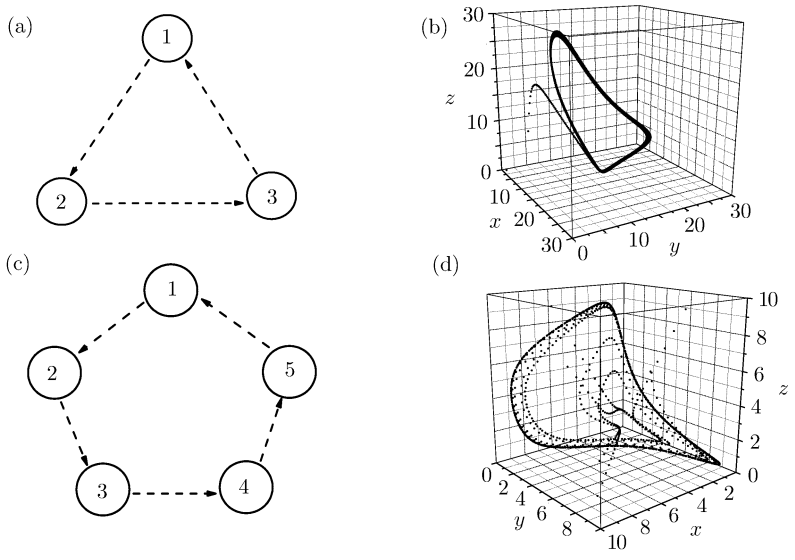

Fig. 2 Oscillations in circuits with odd numbers of genes repressing one another successively. (a) Diagram of the circuit with three repressors. (b) 3-D phase diagram at parameters: $\mu = 0.0$, $\kappa = 0.3$, $\nu = 2.0$, $\gamma = 0.0001$, $\alpha_j^{(i)} = \beta_j^{(i)} = 1.0$, $i, j = 1, 2, 3$. (c) Diagram of the circuit with five repressors. (d) 3-D phase diagram of the 5-gene circuit shown in (c) at parameters: $\mu = 0.0$, $\kappa = 0.3$, $\nu = 2.0$, $\gamma = 0.1$, $\alpha_j^{(i)} = \beta_j^{(i)} = 1.0$, $i, j = 1, 2, 3$.

M. Elowitz and S. Leibler[15] experimentally constructed an autonomous 3-gene TR circuit with three mutually transcriptional repressors showing oscillatory expression variation, and this experimental result is confirmed by our tests. The TR structure is shown in Fig. 2(a), which is one of the four figures in which we found periodic oscillation by exhaustively testing 3-gene TR figures. We present the periodic oscillation of circuit Fig. 2(a) in Fig. 2(b) at the given parameter set. For this symmetric successive repressing circuit we can exactly compute its symmetric steady solution, and can explicitly predict the parameter regions where the steady solution is stable and unstable (associated with stable oscillation). Oscillations can be revealed in all circuits with odd numbers of repressors, precisely, n-gene ($n = 2m+1$, $m = 1, 2, \ldots$) circuits with successive repressive actions, among which the 5-gene circuit and its oscillatory expression variations are shown in Figs. 2(c) and 2(d), respectively.

It is found that the oscillations shown in Figs. 2(b) and 2(d) are sensitive to structure changes of figures. For instance, if we add one more protein to Fig. 2(a) with very weak couplings (Figs. 3(a), 3(b), 3(c)) or change the repression intensity of any single gene in Fig. 2(c) (Figs. 3(d), 3(e), 3(f)), the oscillations of Figs. 2(a) and 2(c) can be reduced to stable focus (Figs. 3(b) and 3(e)) and stable node (Figs. 3(c) and 3(f)) points as the changes increase.

In this letter we investigated dynamic behaviors of 3-, 4-, 5-, 10-gene circuits in detail, and found seldomness of oscillatory motions and nonchaoticity after more than 100 000 tests. Absolutely most of asymptotic solutions of TR circuits described by ODEs of Eq. (1) are steady states. These results are strange since from intuitive expectation oscillations and chaos would be easily observable in high-dimensional ($n \geq 3$) and nonlinear ODEs like Eq. (1).

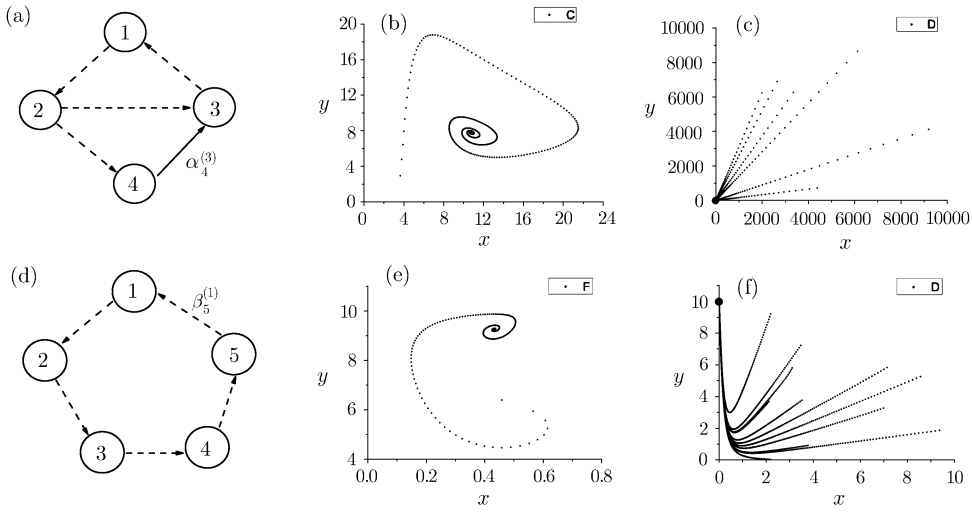

Fig. 3 Sensitivity of oscillations to structure changes of circuits, and collapses of oscillations shown in Fig. 2 by varying the figure structures. (a) A new gene (gene 4) is added to the circuit of Fig. 2(a) and repressed by gene 2. At the same time, it activates gene 3. All parameters are same as Fig. 2(b) with $\alpha_4^{(3)}$ being adjustable. If $\alpha_4^{(3)} = 0.0$ the behavior of Fig. 2(b) is not changed. (b) and (c) Phase diagrams of Fig. 3(a) for different $\alpha_4^{(3)}$'s. (b) A stable focus is observed for $\alpha_4^{(3)} = 0.000\,25$. (c) A stable node appears for $\alpha_4^{(3)} = 0.001$. In both (b) and (c) the oscillatory cycle of Fig. 2(b) collapses to stable steady solutions. (d) The same as Fig. 2(c) with heterogeneity being introduced to a single repression intensity. Precisely, we adjust $\beta_5^{(1)}$ and keep all other parameters same as Fig. 2(b). (e) and (f) The same as Figs. 3(b) and 3(c), respectively, with circuit Fig. 3(d) considered. (e) $\beta_5^{(1)} = 0.08$, (f) $\beta_5^{(1)} = 0.01$.

However, these results coincide with the observations of actual few-gene biological TR circuits, and they are appreciated for normal functions of TR circuits because they provide evidences favorable to stability and controllability of biological TR networks. The dynamic mechanism underlying the seldomness of oscillations and nonchaoticity of Eq. (1) is not yet understood. We found that most of asymptotic states of Eq. (1) are stable nodes rather than focuses. This observation indicates possible weakness of circulation of Eq. (1) for most of network structures, and this may be the main reason for the seldomness of oscillations. Since chaoticity can be regarded to appear via interactions between various intrinsic oscillations, it is thus even more difficult for observing chaos in Eq. (1). We should emphasize that the conjecture of nonchaoticity of ODEs with the TR structure of Eq. (1) has neither been mathematically proven and nor been exhaustively confirmed. However, from our study we can draw an interesting as well as useful conclusion that the probability for the ODEs of Eq. (1) to have chaoticity is extremely low, and this conclusion is favorable for normal TR functions of biological networks.

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