Vol 16 No 9, September 2007
1009-1963/2007/16(09)/2587-08
Chinese Physics
© 2007 Chin. Phys. Soc.
and IOP Publishing Ltd

Dynamics of network motifs in genetic regulatory networks*

Li Ying(李 莹)[a)†], Liu Zeng-Rong(刘曾荣)[b)‡], and Zhang Jian-Bao(张建宝)[a)]

[a)] *Department of Mathematics, Shanghai University, Shanghai* 200444, *China*

[b)] *Institute of Systems Biology, Shanghai University, Shanghai* 200444, *China*

(Received 6 January 2007; revised manuscript received 19 March 2007)

Network motifs hold a very important status in genetic regulatory networks. This paper aims to analyse the dynamical property of the network motifs in genetic regulatory networks. The main result we obtained is that the dynamical property of a single motif is very simple with only an asymptotically stable equilibrium point, but the combination of several motifs can make more complicated dynamical properties emerge such as limit cycles. The above-mentioned result shows that network motif is a stable substructure in genetic regulatory networks while their combinations make the genetic regulatory network more complicated.

Keywords: genetic regulatory network, motif, feedback loop
PACC: 0500, 0547

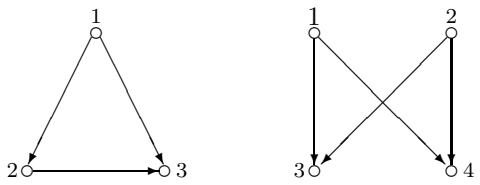
1. Introduction

Bio-molecular networks are often modular in nature, with groups of individual nodes collaborating to carry out some specific biological functions. This has led researchers to investigate more closely the hierarchical structure of real biological interaction networks, and to provide biological explanations for how the observed structure of such networks has emerged. Recently, a loose hierarchical structure for bio-molecular networks has been proposed in Refs.[1,2]. The lowest level in this hierarchy consists of individual nodes, which are then organized into so-called network motifs, small patterns of interconnections occurring in complex networks with numbers significantly higher than those in randomized networks. These are in turn grouped into larger modules of functionally related nodes before the modules are finally connected themselves to form the overall network. Such network motifs have been found in networks of biochemistry, neurobiology, ecology, and engineering.[3] From then on, many kinds of researches have been carried out which become an important aspect of network research. Motifs shared by different networks are distinct from each other; besides, they can define a broad class of networks, each with specific types of elementary structures. Motifs reflect the underlying processes that generate each type of network in a degree.

Transcription networks are biochemical networks responsible for regulating the expression of genes in cells.[4−6] These are directed graphs, in which the nodes represent genes. Edges are directed from a gene that encodes for a transcription factor protein to a gene transcriptionally regulated by that transcription factor. Little is known about the design principles[7−11] of transcriptional regulation networks that control gene expression in cells. Recent advances in data collection and analysis,[12−14] however, are generating unprecedented amounts of information about gene regulation networks. To understand these complex wiring diagrams,[7−11,15] we have sought to break down such networks into basic building blocks,[12] which are the 'network motifs' of transcription regulation networks. Each network motif has a specific function in determining gene expression, such as generating temporal expression programs and governing the responses to fluctuating external signals. The motif structure also allows an easily interpretable view of the entire known transcriptional network of the organism. Milo *et al*[3] analysed the two best characterized transcriptional regulation networks corresponding to organisms in different kingdoms: an enkaryote (the yeast Saccharomyces cerevisiae)[16] and a bacterium (Escherichia coli).[4−6] The two transcription networks show the same motifs: a three-node mo-

*Project supported by the National Natural Science Foundation of China (Grant No 10672093) and Innovation Foundation of Shanghai University for Postgraduates, China.
†E-mail: leepanda@163.com
‡Corresponding author. E-mail: zrongliu@online.sh.cn

tif termed 'feed-forward loop' and a four-node motif termed 'bi-fan'[4] (Fig.1).



(a) the feed-forward loop (b) the bi-fan

Fig.1. The motifs of genetic regulatory networks.

The feed-forward loop and bi-fan motifs have been found in transcriptional regulatory networks and neuron networks, both of which involve some form of information processing.[5] Also, the motifs found in the food-webs studied are distinct from those found in transcriptional regulatory networks and the WWW networks, while electronic circuits with distinct functions tend to have different sets of motifs. These observations have led to the suggestion that there is a connection between a network's motif and its function, and hence, complex networks may be classified into different functional families based on their typical motifs. This shows that the two kinds of motifs play special roles in genetic regulatory networks. Understanding these roles is an important subject for interpreting the behaviour of gene regulation. But there has been little work carried out to analyse their functions from dynamics. We think it is an important aspect to reveal the behaviour of the motifs from the dynamical viewpoint.

From the dynamical behaviour viewpoint,[17−22] however, how to appropriately represent real genetic regulatory systems mathematically in terms of gene function, expression mechanisms, and signal-transduction pathways remains unclear. Mathematical models are useful for discovering higher order structure of an organism and for gaining deep insights into both static and dynamic behaviours of gene networks by extracting functional information from observation data. There are two basic models for genetic networks:[23,24] 1) the Boolean or logical model and 2) the differential equation or dynamic system model. With the Boolean model, the activity of each gene is expressed in one of only two states, ON or OFF, and the state of a gene is determined by a Boolean function of the states of the other related genes. In contrast, the differential equation or dynamic system model describes the concentrations of gene products, such as mRNAs and proteins, as continuous values, which are more accurate and can provide detailed information of the nonlinear dynamical behaviour exhibited by biological systems. There also exist hybrid models that combine discrete and continuous system models and illustrate both switch-like and smooth variations of genetic networks.[25,26]

This paper intends to provide the differential equation models for the motifs of genetic regulatory networks, then analyses their local stability and bifurcation and gives the numerical simulation. By theoretical analysis, we show that the dynamics of a single motif is very simple with only an asymptotically stable equilibrium point. This verifies the fact that a motif is a stable substructure in genetic regulatory networks and occurs more often. Considering that the whole gene regulatory network can express more complicated dynamical properties such as limit cycles, we guess that the combinations of motifs may cause more complicated properties to emerge, which is also validated in this paper.

2. Differential models and dynamical analysis of network motifs

In the first place, we will introduce the delay differential equation (DDE) model for genetic regulatory networks by exploiting the structure of the genome-proteome network and by representing mRNAs and proteins with different variables proposed by Chen as follows:[27]

$$\dot{m}(t) = -K_m m(t) + c(p(t, \tau_p)), \tag{1}$$

$$\dot{p}(t) = -K_p p(t) + d(m(t, \tau_m)), \tag{2}$$

where

$$m = (m_1, m_2, \ldots, m_n) \in R^n$$

and

$$p = (p_1, p_2, \ldots, p_n) \in R^n$$

represent the concentrations of mRNAs and proteins, respectively. $K_m = \mathrm{diag}(k_{m1}, \ldots, k_{mn}) \in R^{n\times n}$ and $K_p = \mathrm{diag}(k_{p1}, \ldots, k_{pn}) \in R^{n\times n}$ are positive real diagonal matrices that represent the degradation rates of mRNAs and proteins, respectively. $\tau_m = (\tau_{m1}, \ldots, \tau_{mn}) \in R^n$ and $\tau_p = (\tau_{p1}, \ldots, \tau_{pn}) \in R^n$ are positive real vectors indicating the delays of mRNAs and proteins respectively, and we have $m(t, \tau_m) = (m_1(t - \tau_{m1}), \ldots, m_n(t - \tau_{mn}))$ and $p(t, \tau_p) = (p_1(t -$

$\tau_{p1}), \ldots, p_n(t-\tau_{pn}))$. $c(p) = (c_1(p), \ldots, c_n(p)) \in R^n$ and $d(m) = (d_1(m_1), \ldots, d_n(m_n)) \in R^n$ are generally nonlinear. Note that d_i is defined not as $d_i(m)$ but as $d_i(m_i)$, in contrast to $c_i(p)$. Generally, in genetic regulatory networks, $(c(x), d(x))$ can be expressed with a sigmoid function, $\tanh(x_i/\epsilon)$ or $x_i^k/(x_i^k+\epsilon)$ or $\alpha/(x_i^k+\epsilon)$, where ϵ and α are positive numbers and k is the Hill coefficient, denoting the degree of cooperativity.

Based on the model of Chen, we propose the differential equation model for network motifs in genetic regulatory networks. In order to simplify our model, we ignore the time delays.

First, we consider the single motifs of 'feed-forward loop' and 'bi-fan' separately. Without losing generality, suppose that all the control signs are negative. Mathematically, dynamics can be described by the following equations for the tree-node motif and the four-node motif respectively:

$$\begin{aligned}
&\dot{m}_1 = -m_1 + \alpha_0,\\
&\dot{p}_1 = -\beta p_1 + \beta m_1,\\
&\dot{m}_2 = -m_2 + \frac{\alpha}{1+p_1^n} + \alpha_0,\\
&\dot{p}_2 = -\beta p_2 + \beta m_2,\\
&\dot{m}_3 = -m_3 + \frac{\alpha}{1+p_1^n} + \frac{\alpha}{1+p_2^n} + \alpha_0,\\
&\dot{p}_3 = -\beta p_3 + \beta m_3; \qquad (3)\\
&\dot{m}_i = -m_i + \alpha_0,\\
&\dot{p}_i = -\beta p_i + \beta m_i, \quad i = 1, 2,\\
&\dot{m}_j = -m_j + \frac{\alpha}{1+p_1^n} + \frac{\alpha}{1+p_2^n} + \alpha_0,\\
&\dot{p}_j = -\beta p_j + \beta m_j, \quad j = 3, 4, \qquad (4)
\end{aligned}$$

where n is the Hill coefficient. All parameters and variables are positive real numbers.

Conclusion 1 The equilibrium points of Eqs.(3) and (4) are both globally asymptotically stable, no matter what values the parameters take.

Proof Take Eq.(3) as an example.

It is easy to validate that Eq.(3) has a unique globally asymptotically stable equilibrium point

$$\begin{aligned}
\bar{P} &= (m_1, m_2, m_3, p_1, p_2, p_3)\\
&= \Big(\alpha_0, \alpha_0, \alpha_0 + \frac{\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{\alpha}{1+\alpha_0^n},\\
&\quad \alpha_0 + \frac{2\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{2\alpha}{1+\alpha_0^n}\Big).
\end{aligned}$$

We can obtain the same conclusion for Eq.(4).

Remark This result also comes into existence in the situations where exist positive regulations and all kinds of parameter values in the motifs.

The key of the theorem's proof is to use the uncoupling property of the dynamical system. The first two equations in Eq.(3) are uncoupled with the last four equations, so the subsystem of the first two equations has a unique globally asymptotically stable equilibrium point $P_1 = (m_1, p_1) = (\alpha_0, \alpha_0)$. Substituting the value into the middle two equations in Eq.(3) which are uncoupled with the last two equations in Eq.(3), we have the corresponding globally asymptotically stable equilibrium point

$$P_2 = (m_2, p_2) = \Big(\alpha_0 + \frac{\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{\alpha}{1+\alpha_0^n}\Big).$$

Similarly, we can have the globally asymptotically stable equilibrium point of the subsystem of the last two equations

$$P_3 = (m_3, p_3) = \Big(\alpha_0 + \frac{2\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{2\alpha}{1+\alpha_0^n}\Big).$$

Finally, we gain the unique globally asymptotically stable equilibrium point

$$\begin{aligned}
\bar{P} &= (m_1, m_2, m_3, p_1, p_2, p_3)\\
&= \Big(\alpha_0, \alpha_0, \alpha_0 + \frac{\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{\alpha}{1+\alpha_0^n},\\
&\quad \alpha_0 + \frac{2\alpha}{1+\alpha_0^n}, \alpha_0 + \frac{2\alpha}{1+\alpha_0^n}\Big).
\end{aligned}$$

Due to this property, we can give an important lemma for genetic regulatory network without feedback loops. Reference [28] gives the detailed definitions of feedback loops and their types (positive feedback loops and negative feedback loops).

Lemma 1 If a genetic regulatory network with finite nodes does not contain the substructure of feedback loops, then its dynamical equation has an asymptotically stable equilibrium point.

Proof Suppose $G = \{V, E\}$ is a genetic regulatory network without feedback loops, where V and E are the sets of nodes and edges of G respectively. Let N be the number of nodes $N = |V|$.

According to the graph theory, if a directed network with finite nodes, without any subgraph of loop, then there is at least one node without inputs. So there is at least one node without inputs in G. Based on the analysis of the proof of Conclusion 1, the effects of these nodes are equivalent to constants. Namely, we can replace the variables of the nodes without inputs

with constants which are coordinates of their asymptotically stable equilibrium points. Thus the dynamical system is simplified, which is equivalent to removing the nodes without inputs and their outputs. The remaining subnetwork is still a directed network without feedback loops. So there is still at least one node without inputs.

Repeating this process, we will have the least network of one isolated node after finite steps. The dynamics of the whole network G is equivalent to that of the isolated node. Naturally, the dynamical equation of the remaining node also has an asymptotically stable equilibrium point.

From this lemma, we have the following conclusion.

Conclusion 2 The dynamics of the subnetwork made up of motifs and containing no loop has a globally asymptotically stable equilibrium point.

Proof It is a direct corollary of Lemma 1.

Based on Conclusion 1, as long as there is no feedback loops in a subnetwork made up of motifs, the dynamics of the subnetwork has a stable equilibrium point without any other complex properties. So we only need to consider the dynamics of the combinations of motifs that contain at least one feedback loop. It is easy to see that any of the combinations of two motifs (the three-node one or the four-node one) does not contain feedback loops no matter how they combine. Only the combination of three or more motifs probably contains feedback loops.

In this paper, we will take the simple combinations of tree motifs which contain feedback loops as examples and analyse their dynamics in detail. The other combinations of more than tree motifs can be analysed similarly. Figure 2 gives the possible combinations of three motifs. Actually, the methods of analysing all of the dynamics in Fig.2 are similar. For a monotone dynamical system, if all of the feedback loops are positive, there will be no limit cycle.[29] For such a system, there are only stable equilibria. If there is one negative feedback loop, the dynamics becomes very complicated, which may be e.g. stable equilibria, stable periodic solution or chaos, depending on the conditions.[29] Thus we have the following conclusion for the subnetworks made up of motifs and containing only positive feedback loops:

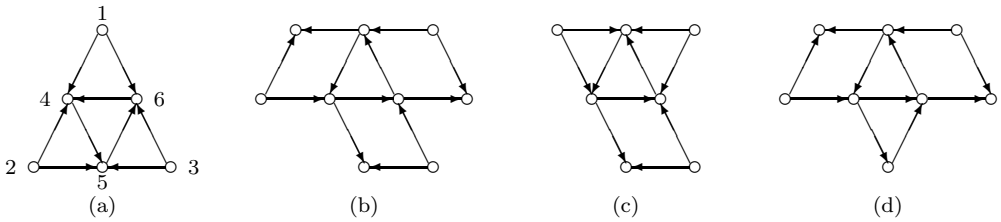


Fig.2. Three motifs' combinations with feedback loop.

Conclusion 3 The dynamics of the subnetwork, which is made up of motifs and containing only positive feedback loops, has stable equilibrium points. But the equilibrium points are not necessarily unique.

According to this conclusion we only need to consider the situations when the subnetwork made up of motifs has a negative feedback loop. Without losing generality, we assume that the regulatory intersections in the same motifs have the same sign. We take Fig.2(a) as an example and analyse its dynamics in two situations when there are negative feedback loops:

1) All the regulatory intersections in the three motifs are negative;

2) The regulatory intersections in the three motifs are different: negative in one motif and positive in the other two motifs.

First, we analyse the dynamics of Fig.2(a) in the first situation.

$$
\begin{aligned}
&\dot{m}_i = -m_i + \alpha_1,\\
&\dot{p}_i = -\beta p_i + \beta m_i, \quad i = 1,2,3,\\
&\dot{m}_4 = -m_4 + \frac{\alpha}{1+p_6^n} + \frac{\alpha_2}{1+p_1^n} + \frac{\alpha_2}{1+p_2^n} + \alpha_3,\\
&\dot{p}_4 = -\beta p_4 + \beta m_4,\\
&\dot{m}_5 = -m_5 + \frac{\alpha}{1+p_4^n} + \frac{\alpha_2}{1+p_2^n} + \frac{\alpha_2}{1+p_3^n} + \alpha_3,\\
&\dot{p}_5 = -\beta p_5 + \beta m_5,\\
&\dot{m}_6 = -m_6 + \frac{\alpha}{1+p_5^n} + \frac{\alpha_2}{1+p_1^n} + \frac{\alpha_2}{1+p_3^n} + \alpha_3,\\
&\dot{p}_6 = -\beta p_6 + \beta m_6.
\end{aligned} \tag{5}
$$

Let us see the subsystem of Eq.(5):

$$
\begin{aligned}
&\dot{m}_i = -m_i + \alpha_1,\\
&\dot{p}_i = -\beta p_i + \beta m_i, \quad i = 1,2,3.
\end{aligned} \tag{6}
$$

Obviously, this subsystem asymptotically converges to its equilibrium point $\bar{P}_0 = (\alpha_1, \alpha_1, \alpha_1, \alpha_1, \alpha_1, \alpha_1)$. So the constant α_1 can replace m_i and p_i $(i = 1, 2, 3)$ in Eq.(5). Thus the original system Eq.(5) is equivalent to the following:

$$\begin{aligned}
\dot{m}_4 &= -m_4 + \frac{\alpha}{1+p_6^n} + \frac{2\alpha_2}{1+\alpha_1^n} + \alpha_3,\\
\dot{p}_4 &= -\beta p_4 + \beta m_4,\\
\dot{m}_5 &= -m_5 + \frac{\alpha}{1+p_4^n} + \frac{2\alpha_2}{1+\alpha_1^n} + \alpha_3,\\
\dot{p}_5 &= -\beta p_5 + \beta m_5,\\
\dot{m}_6 &= -m_6 + \frac{\alpha}{1+p_5^n} + \frac{2\alpha_2}{1+\alpha_1^n} + \alpha_3,\\
\dot{p}_6 &= -\beta p_6 + \beta m_6. \qquad (7)
\end{aligned}$$

In order to analyse the dynamics of Eq.(5), we just need to study the dynamical property of Eq.(7).

When all variables are positive, clearly there is a unique equilibrium point $P_0 = (p, p, p, p, p, p)$ with identical elements, where $p > 0$ and

$$p = \frac{\alpha}{1+p^n} + \frac{2\alpha_2}{1+\alpha_1^n} + \alpha_3. \qquad (8)$$

The Jacobin matrix of Eq.(7) at the equilibrium point P_0 is

$$J_1 = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & X \\ \beta & -\beta & 0 & 0 & 0 & 0 \\ 0 & X & -1 & 0 & 0 & 0 \\ 0 & 0 & \beta & -\beta & 0 & 0 \\ 0 & 0 & 0 & X & -1 & 0 \\ 0 & 0 & 0 & 0 & \beta & -\beta \end{bmatrix}, \qquad (9)$$

where $X = -\alpha n p^{n-1}/(1+p^n)^2$, and p satisfies Eq.(8).

Hence, the eigenvalues of J_1 at P_0 satisfy the following equation:

$$[(\lambda+1)(\lambda+\beta)]^3 = (X\beta)^3. \qquad (10)$$

Let $\gamma_i^3 = (X\beta)^3$, then $\gamma_1 = -\beta X \mathrm{e}^{\mathrm{j}\pi/3}$, $\gamma_2 = -\beta X \mathrm{e}^{\mathrm{j}\pi}$, $\gamma_3 = -\beta X \mathrm{e}^{5\mathrm{j}\pi/3}$.

That is

$$\gamma_i = (\lambda+1)(\lambda+\beta). \qquad (11)$$

When $i = 2$, $(\lambda+1)(\lambda+\beta) = \beta X$, that is,

$$\lambda = \frac{-(1+\beta) \pm \sqrt{(1+\beta)^2 - 4\beta(1-X)}}{2}$$

has negative real parts.

Next we analyse the conditions for $i = 1, 3$.

Let $\gamma_i = u_i + v_i\mathrm{j}$ and $\lambda = u + v\mathrm{j}$. We will derive the condition for $u < 0$, $u = 0$ and $u > 0$ separately.

Substituting the expressions of γ_i and λ, we have the following equations:

$$u^2 + (1+\beta)u + \beta - u_i = v^2, \qquad (12)$$

$$(2u + 1 + \beta)v = v_i. \qquad (13)$$

Define

$$\begin{aligned}
y_{\mathrm{L}}(u) &= u^2 + (1+\beta)u + \beta - u_i,\\
y_{\mathrm{R}}(u) &= \frac{v_i^2}{(2u+1+\beta)^2}, \qquad (14)
\end{aligned}$$

where y_{L} is the left-hand of Eq.(12) and y_{R} is the right-hand side of Eq.(12) obtained by substituting v of Eq.(13) in it.

By analysing graphically y_{L} and y_{R} with variable u, we can have the following results.

1) The sufficient and necessary condition of two negative real roots for $y_{\mathrm{L}}(u) = y_{\mathrm{R}}(u)$ is

$$\beta - u_i > \frac{v_i^2}{(1+\beta)^2},$$

where the left-hand side and the right-hand side correspond to the values of y_{L} and y_{R} at $u = 0$ respectively. Substituting u_i and $v_i (i = 1, 3)$ into the above equation, we obtain

$$\frac{(1+\beta)^2}{\beta}(4 + 2X) > 3X^2.$$

2) Similarly, the sufficient and necessary condition of one negative real root and one zero root for $y_{\mathrm{L}}(u) = y_{\mathrm{R}}(u)$ is

$$\frac{(1+\beta)^2}{\beta}(4 + 2X) = 3X^2.$$

3) The sufficient and necessary condition of at least one positive root for $y_{\mathrm{L}}(u) = y_{\mathrm{R}}(u)$ is

$$\frac{(1+\beta)^2}{\beta}(4 + 2X) < 3X^2.$$

From the analysis above, we can obtain the following conclusion naturally.

Conclusion 4 If

$$\frac{(1+\beta)^2}{\beta}(4 + 2X) > 3X^2,$$

then the equilibrium point of Eq.(5) is asymptotically stable; if

$$\frac{(1+\beta)^2}{\beta}(4 + 2X) = 3X^2,$$

then there is Hopf bifurcation, where $X = -\alpha n p^{n-1}/(1+p^n)^2$ and p satisfies Eq.(8).

Next, we analyse the second situation, i.e. the regulatory intersections in the three motifs are different. Suppose the regulatory intersections in the motif

of nodes $\{3,5,6\}$ are negative and the others are positive. The model is expressed as

$$\begin{aligned}
&\dot{m}_i = -m_i + \alpha_1,\\
&\dot{p}_i = -\beta p_i + \beta m_i, \quad i = 1,2,3,\\
&\dot{m}_4 = -m_4 + \frac{\alpha p_6^n}{1+p_6^n} + \frac{\alpha_2 p_1^n}{1+p_1^n} + \frac{\alpha_2 p_2^n}{1+p_2^n} + \alpha_3,\\
&\dot{p}_4 = -\beta p_4 + \beta m_4,\\
&\dot{m}_5 = -m_5 + \frac{\alpha p_4^n}{1+p_4^n} + \frac{\alpha_2 p_2^n}{1+p_2^n} + \frac{\alpha_2}{1+p_3^n} + \alpha_3,\\
&\dot{p}_5 = -\beta p_5 + \beta m_5,\\
&\dot{m}_6 = -m_6 + \frac{\alpha}{1+p_5^n} + \frac{\alpha_2 p_1^n}{1+p_1^n} + \frac{\alpha_2}{1+p_3^n} + \alpha_3,\\
&\dot{p}_6 = -\beta p_6 + \beta m_6.
\end{aligned} \tag{15}$$

The equivalent system is

$$\begin{aligned}
&\dot{m}_4 = -m_4 + \frac{\alpha p_6^n}{1+p_6^n} + \frac{2\alpha_2 \alpha_1^n}{1+\alpha_1^n} + \alpha_3,\\
&\dot{p}_4 = -\beta p_4 + \beta m_4,\\
&\dot{m}_5 = -m_5 + \frac{\alpha p_4^n}{1+p_4^n} + \frac{\alpha_2}{1+\alpha_1^n} + \frac{\alpha_2 \alpha_1^n}{1+\alpha_1^n} + \alpha_3,\\
&\dot{p}_5 = -\beta p_5 + \beta m_5,\\
&\dot{m}_6 = -m_6 + \frac{\alpha}{1+p_5^n} + \frac{\alpha_2 \alpha_1^n}{1+\alpha_1^n} + \frac{\alpha_2}{1+\alpha_1^n} + \alpha_3,\\
&\dot{p}_6 = -\beta p_6 + \beta m_6.
\end{aligned} \tag{16}$$

Denote the equilibrium point of Eq.(16) as $P = (\bar{m}_4, \bar{p}_4, \bar{m}_5, \bar{p}_5, \bar{m}_6, \bar{p}_6)$. At point P, the Jacobin matrix is

$$J_2 = \begin{bmatrix}
-1 & 0 & 0 & 0 & 0 & X_1\\
\beta & -\beta & 0 & 0 & 0 & 0\\
0 & X_2 & -1 & 0 & 0 & 0\\
0 & 0 & \beta & -\beta & 0 & 0\\
0 & 0 & 0 & X_3 & -1 & 0\\
0 & 0 & 0 & 0 & \beta & -\beta
\end{bmatrix}, \tag{17}$$

where

$$X_1 = \frac{\alpha n \bar{p}_6^{n-1}}{(1+\bar{p}_6^n)^2} > 0, \quad X_2 = \frac{\alpha n \bar{p}_4^{n-1}}{(1+\bar{p}_4^n)^2} > 0,$$

$$X_3 = -\frac{\alpha n \bar{p}_5^{n-1}}{(1+\bar{p}_5^n)^2} < 0.$$

Hence, the eigenvalues of J_2 satisfy

$$[(\lambda+1)(\lambda+\beta)]^3 = \beta^3 X_1 X_2 X_3. \tag{18}$$

Let $\gamma_i^3 = (X\beta)^3$, then

$$\gamma_1 = -\beta \sqrt[3]{X_1 X_2 X_3} \mathrm{e}^{\mathrm{j}\pi/3}, \quad \gamma_2 = -\beta \sqrt[3]{X_1 X_2 X_3} \mathrm{e}^{\mathrm{j}\pi},$$

$$\gamma_3 = -\beta \sqrt[3]{X_1 X_2 X_3} \mathrm{e}^{5\mathrm{j}\pi/3}.$$

That is

$$\gamma_i = (\lambda+1)(\lambda+\beta). \tag{19}$$

For γ_2, the roots of Eq.(19) are

$$\lambda_{1,2} = \frac{-(1+\beta) \pm \sqrt{(1+\beta)^2 - 4\beta(1-\sqrt[3]{X_1 X_2 X_3})}}{2}.$$

According to $\sqrt[3]{X_1 X_2 X_3} \le 0$, $\lambda_{1,2} < 0$.

For $\lambda_{1,3}$, we have

$$y_{\mathrm{L}}(0) = \beta\Big(1 + \frac{1}{2}\sqrt[3]{X_1 X_2 X_3}\Big),$$

$$y_{\mathrm{R}}(0) = \frac{3}{4}\frac{\beta^2 (\sqrt[3]{X_1 X_2 X_3})^2}{(1+\beta)^2}.$$

1) When $y_{\mathrm{L}}(0) > y_{\mathrm{R}}(0)$, that is

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) > 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

all of the eigenvalues of J_2 have negative real parts. In this case, the equilibrium point is asymptotically stable.

2) When $y_{\mathrm{L}}(0) = y_{\mathrm{R}}(0)$, that is

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) = 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

J_2 has a pair of pure imaginary eigenvalues and the other eigenvalues have negative real parts. Here Hopf-bifurcation occurs.

3) When $y_{\mathrm{L}}(0) < y_{\mathrm{R}}(0)$, that is

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) < 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

some of the eigenvalues of J_2 have positive real parts, which indicate the unstable equilibrium points.

Conclusion 5 If

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) > 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

then the equilibrium point of Eq.(15) is asymptotically stable; if

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) = 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

then there is Hopf bifurcation; if

$$\frac{(1+\beta)^2}{\beta}(4 + 2\sqrt[3]{X_1 X_2 X_3}) < 3(\sqrt[3]{X_1 X_2 X_3})^2,$$

there is at least one unstable equilibrium point, where

$$X_1 = \frac{\alpha n \bar{p}_6^{n-1}}{(1+\bar{p}_6^n)^2} > 0, \quad X_2 = \frac{\alpha n \bar{p}_4^{n-1}}{(1+\bar{p}_4^n)^2} > 0,$$

$$X_3 = -\frac{\alpha n \bar{p}_5^{n-1}}{(1+\bar{p}_5^n)^2} < 0.$$

Remark The situation we discuss here is a little special. For more general situations, we can also show the analysis similarly and obtain the similar results just with more parameters and more complicated expressions.

3. Numerical simulation

In Section 2, we have given the theoretical analysis. In order to illustrate our theoretical result, we take the parameters in Eq.(5) as follows: $n = 2$, $\beta = 1$, $\alpha = 4.28$, $\alpha_1 = 1$, $\alpha_2 = 0.003$, $\alpha_3 = 0.00128$. According to numerical calculation, the inequality

$$\frac{(1+\beta)^2}{\beta}(4+2X) < 3X^2$$

holds, where $X = -(\alpha n p^{n-1}/(1+p^n)^2$ and p satisfies Eq.(8). In other words, Eq.(7) has a limit cycle. The time evolution of $(m_i(t),\ p_i(t))$, $i = 4, 5, 6$ of Eq.(5) are shown in Fig.3, which indicates the limit cycle of Eq.(7). Similarly, we take $n = 8$, $\beta = 1$, $\alpha = 1.34$, $\alpha_1 = 1$, $\alpha_2 = 0.6$, $\alpha_3 = 0.06$ in Eq.(16) which satisfy the inequality

$$\frac{(1+\beta)^2}{\beta}(4+2\sqrt[3]{X_1X_2X_3}) < 3(\sqrt[3]{X_1X_2X_3})^2,$$

where

$$X_1 = \frac{\alpha n \bar{p}_6^{n-1}}{(1+\bar{p}_6^n)^2} > 0, \quad X_2 = \frac{\alpha n \bar{p}_4^{n-1}}{(1+\bar{p}_4^n)^2} > 0,$$

$$X_3 = -\frac{\alpha n \bar{p}_5^{n-1}}{(1+\bar{p}_5^n)^2} < 0.$$

In Fig.4, the time evolutions of $(m_i(t),\ p_i(t))$ are shown to be periodic, where $i = 4, 5, 6$.

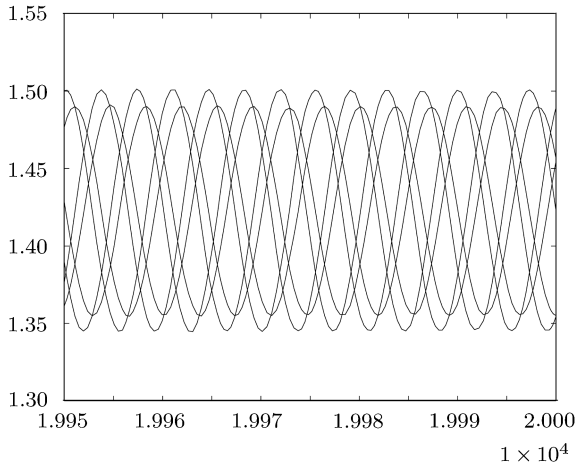


Fig.3. Time evolution of $(m_i(t), p_i(t))$ $(i = 4, 5, 6)$ of system Eq.(7), with $n = 2$, $\beta = 1$, $\alpha = 4.28$, $\alpha_1 = 1$, $\alpha_2 = 0.003$, $\alpha_3 = 0.00128$.

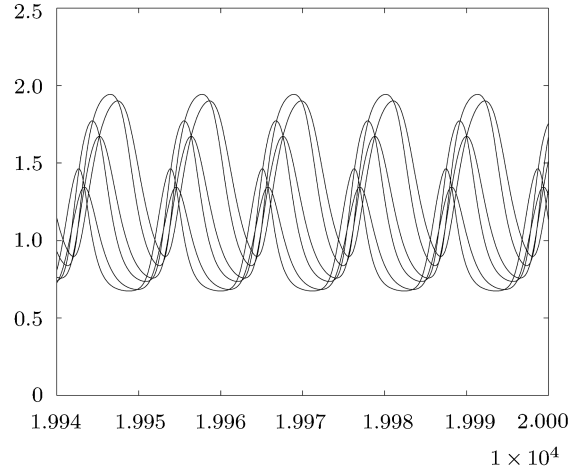


Fig.4. Time evolution of $(m_i(t), p_i(t))$ $(i = 4, 5, 6)$ of system Eq.(16), with $n = 8$, $\beta = 1$, $\alpha = 1.34$, $\alpha_1 = 1$, $\alpha_2 = 0.6$, $\alpha_3 = 0.06$.

4. Conclusion

In this paper, we have analysed the dynamics of genetic regulatory motifs. We show that as long as there is no feedback loop in a subnetwork made up of genetic regulatory motifs, the dynamics of the network has a stable equilibrium point without any other complex properties. Moreover, we theoretically derive the sufficient conditions for the dynamics of motifs' combinations containing a negative feedback loop to have a limit cycle. The simulation confirms the theoretical results.

Our result illustrates that the network motif is a stable substructure in genetic regulatory networks. Moreover, the combinations of the simple motifs can make the genetic regulatory network have more complicated dynamical properties.

Recent progress in genetic engineering has made the design and implementation of synthetic gene networks realistic from both theoretical and experimental viewpoints. Designing and implementing synthetic gene networks provide a natural framework for reducing the complexity of gene regulation. That is to say, the aim of synthetic biology is to synthesize some elements, which have important biological meaning, using the most basic gene regulatory process. And these elements can be achieved for not only engineering but also organisms. So the work of this paper provides a theoretical basis for the synthetic gene networks by taking motifs as the basic elements. The detailed research will be carried out in the future.

References

[1] Babu M M, Luscombe N M, Aravind L, Gerstein M and Teichmann S A 2004 *Curr. Opin. Struct. Biol.* **14** 283

[2] Barabasi L and Oltvai Z 2004 *Nat. Rev. Genet.* **5** 101

[3] Milo R, Shen-Orr S and Itzkovitz S 2002 *Science* **298** 824

[4] Shen-Orr S, Milo R, Mangan S and Alon U 2002 *Nat. Genet.* **31** 64

[5] Lee T *Science* **298** 799

[6] Thieffry D, Huerta A M, Perez-Rueda E and Collado-Vides J 1998 *Bioessays* **20** 433

[7] Bray D 1995 *Nature* **376** 307

[8] Thieffry D, Huerta A M, Pere-Rueda E and Collada-Vides J 1998 *Bioessays* **20** 433

[9] McAdams H H and Shapiro L 1995 *Science* **269** 650

[10] Rao C V and Arkin A P 2001 *Annu. Rev. Biomed. Eng.* **3** 391

[11] Barabasi A L and Albert R 1999 *Science* **286** 509

[12] Hartwell L H, Hopfield J J, Leibler S and Murray A W 1998 *Nature* **402** C47

[13] Hughes J D, Estep P W, Tavazonie S and Church G M 2000 *J. Mol. Biol.* **296** 1205

[14] Hartemink A J, Gifford D K, Jaakkola T S and Yong R A 2001 *Proc. Symp. Biocomputing* (in Hawaii) p422

[15] Newman M E, Strogatz S H and Watts D J 2001 *Phys. Rev.* E **64** 026118

[16] Costanzo M C, Crawford M E, Hirschman J E and Kranz J E 2001 *Nucleic Acids Res.* **29** 75

[17] Luo S Y, Shao M Z, Wei L X and Liu Z R 2004 *Acta Phys. Sin.* **53** 1940 (in Chinese)

[18] Wu J J, Gao Z Y and Sun H J 2007 *Chin. Phys.* **16** 47

[19] Gao T G, Chen Z Q, Chen G R and Yuan Z Z 2006 *Chin. Phys.* **15** 1190

[20] Wang M S, Hou Z H and Xin H W 2006 *Chin. Phys.* **15** 2553

[21] Song Y Z, Zhao G Z and Qi D L 2006 *Chin. Phys.* **15** 2266

[22] Ma S J, Xu W, Li W and Fang T 2006 *Chin. Phys.* **15** 1231

[23] Smolen P, Baxter D A and Byrne J H 2000 *Neuron* **26** 567

[24] Chen T, He H L and Church G M 1999 *Pac. Symp. Biocomputing* (in Hawaii) p29

[25] McAdams H and Shapiro L 1995 *Science* **269** 650

[26] Chen L and Aihara K 2001 *IEEE Trans. Circuits* I **48** 308

[27] Chen L and Aihara K 2002 *IEEE Trans. Circuits* I **49**(5) 602

[28] Tetsuya K, Chen L and Kazuyuki A 2003 *J. Theor. Biol.* **221** 379

[29] Smith H L 1995 *Monotone Dynamical Systems* **41** (Providence, RI: American Mathematical Society) p31