Oscillatory and anti-oscillatory motifs in genetic regulatory networks*

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Recently, self-sustained oscillatory genetic regulatory networks (GRNs) have attracted significant attention in the biological field. Given a GRN, it is important to anticipate whether the network could generate oscillation with proper parameters, and what the key ingredients for the oscillation are. In this paper the ranges of some function-related parameters which are favorable to sustained oscillations are considered. In particular, some oscillatory motifs appearing with high-frequency in most of the oscillatory GRNs are observed. Moreover, there are some anti-oscillatory motifs which have a strong oscillation repressing effect. Some conclusions analyzing these motif effects and constructing oscillatory GRNs are provided.

Keywords: genetic regulatory network, oscillatory motif, anti-oscillatory motif, feedback loop

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1. Introduction

In biological systems, oscillations of gene expression have been found in many different physiological processes. It plays an essential role in a variety of living organisms.[1−13] Since the genomes of many organisms have been sequenced, various dynamic cellular behaviors have been successfully modeled in terms of elementary circuitries. Many researchers have investigated the self-sustained oscillatory genetic regulatory networks (GRNs).[14−16] Thirty years ago, Thomas conjectured that at least one negative feedback loop (i.e., a loop with an odd number of repressors) is needed for GRNs to have periodic oscillating behavior, and at least one positive feedback loop is needed for GRNs to have multistability (i.e., existence of multiple steady states), which had been rigorously proven in a monotone system by Snoussi and Plahte,[17,18] Thomas also pointed out that negative feedback loops in interacting genomic circuits either lead the system dynamics to a stable fixed point, corresponding to the biological concept of homeostasis, or generate stable oscillation.[19] Joseph found that in Cdc2/APC systems, short-circuiting positive feedbacks make oscillations in Cdc2 activity faster, less temporally abrupt, and damped.[20] Goh conjectured that the network could generate oscillation as a result of the competition between positive and negative feedback loops.[21] In 2000, Elowitz constructed a biological 3-node autonomous circuit where oscillation of a biological GRN was indeed experimentally observed for the first time.[14]

Lots of up to date data are accessible, protein concentrations and reaction rates can often be quantified with satisfactory precision so that we are able to use ordinary differential equations (ODEs) to model GRNs. Oscillation is important for various organisms and it appears in lots of GRNs. Therefore, it is significant to study where we can find and how we can effectively construct oscillatory GRNs. For example the following questions should be answered: is there some relation between oscillation and control parameters, and if so, what is the relation? In this paper, we perform large numbers of tests for $4, 5$-node GRNs with different topologically distinctive networks. We find that there is a range of parameters favorable to generate oscillation.

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More interestingly, we undertake a systematic investigation of the characteristic structures favorable to the oscillation of GRNs. First, the probability that the number of negative feedback loops is greater than the number of positive feedback loops is rather large. Second, there are some oscillatory motifs appearing with high frequency. These characteristics are useful for us to predict, with high probability, whether different types of GRNs could generate oscillation. We investigate all oscillatory q-node motifs of GRNs for $q \leq 5$, and find that a few kinds of oscillatory motifs are very useful for generating oscillation. Moreover, if we combine properly some oscillatory motifs, the probability of generating oscillation can be greatly enhanced. Finally, we also find that there are some anti-oscillatory motifs which have a strong repressing effect on oscillation.

2. Model and methods

We use a set of ODEs widely used for describing genetic cis-regulation,[15,22] which adopt an "OR" gate between all repressive and active regulations. Typical mathematical formulas of GRNs of this type with size N are given as follows:

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \gamma_i(1 - x_i) + \sum_{j=1}^{N} f_{ij}, \quad i = 1, 2, \ldots, N, \quad (1)$$

where

$$f_{ij} = \begin{cases} 0, & \text{no coupling,} \\ (1 - x_i)\dfrac{{x_j}^{h_{ij}}}{K_{ij}^{h_{ij}} + {x_j}^{h_{ij}}}, & \text{active coupling,} \\ -x_i\dfrac{{x_j}^{h_{ij}}}{K_{ij}^{h_{ij}} + {x_j}^{h_{ij}}}, & \text{repressive coupling,} \end{cases}$$

and x_i represents the concentration of the i-th protein. There are three parameters in Eq. (1). K is the half-maximal concentration of activator (repressor); h stands for the Hill coefficient and γ represents the decay rate. For mathematical simplicity we take identical parameters in Eq. (1) for all nodes $K_{ij} = K$, $h_{ij} = h$, $\gamma_i = \gamma$, $i, j = 1, 2, \ldots, N$.

Factor x_i in f_{ij} appears only in negative regulation, because the greater the concentration of the regulated node, the stronger the negative regulation. On the other hand, factor $1 - x_i$ in f_{ij} is only for the positive regulation, because the greater the concentration of the regulated node, the weaker the effect of positive regulation (in the saturated case $x(i) = 1$, the positive regulation will no longer increase x_i).

3. Numerical results

3.1. Influence of system parameters on oscillatory behaviors of GRNs

We compute ODE Eq. (1) for few-gene networks, where auto-regulations are permitted, while external stimuli are excluded (we consider autonomous network dynamics). Networks containing isolated nodes are also ruled out from our sets.

We made 10 thousand tests for each of both 4- and 5-node GRNs with different topologically distinctive networks, and we searched the oscillatory range in the parameter intervals currently accepted by biologists (i.e., $K \in [0.1, 2.0]$, $h \in [1, 10]$, $\gamma \in [0.01, 0.25]$). We find that larger h, smaller γ, and middle range of K are favorable for GRN oscillation. Note, suitable parameters are necessary but not solely sufficient for oscillation conditions. In Fig. 1(a), we choose an autonomous GRN with three successive repressive interactions (i.e., repressilator,[14]) which so far has been investigated extensively, and then we scan its parameter domain for oscillation in Figs. 1(b)–1(d). The shadow areas represent the parameter regions where some initial conditions can evolve to asymptotic network oscillation, while in the blank areas all initial conditions approach steady states. From Figs. 1(b)–1(d), we find that small γ and large h favor a high probability of oscillation, while some intermediate value K could produce oscillation.

In order to explore bifurcations of the GRN system to oscillation, we perform linear stability analysis of the steady state solution of the repressilator for different system parameters. In Figs. 2(a)–2(c), we plot the graph of the largest real part of the eigenvalues of the linear matrix of the steady solution of Eq. (1) for network Fig. 1(a). It is found that the maximum real part of the eigenvalues rises as h rises, and declines as γ rises. By changing various parameters, we observe $\lambda_{\max}$ curves crossing zero, where super critical Hopf bifurcations occur. In Figs. 2(d) and 2(e), we plot the corresponding Hopf bifurcations. Before Hopf bifurcation, solid lines denote the stable steady state of the system. After the Hopf bifurcation, the two solid lines correspond to the maximum and minimum levels of concentration $x(1)$ during the oscillation. Figure 2 shows that in the region of oscillation increasing (decreasing) h (γ) makes the amplitude increase, and the range of $K \approx 0.6$ corresponds to the largest oscillation amplitude that coincides approximately with the position of the peak in Fig. 1.

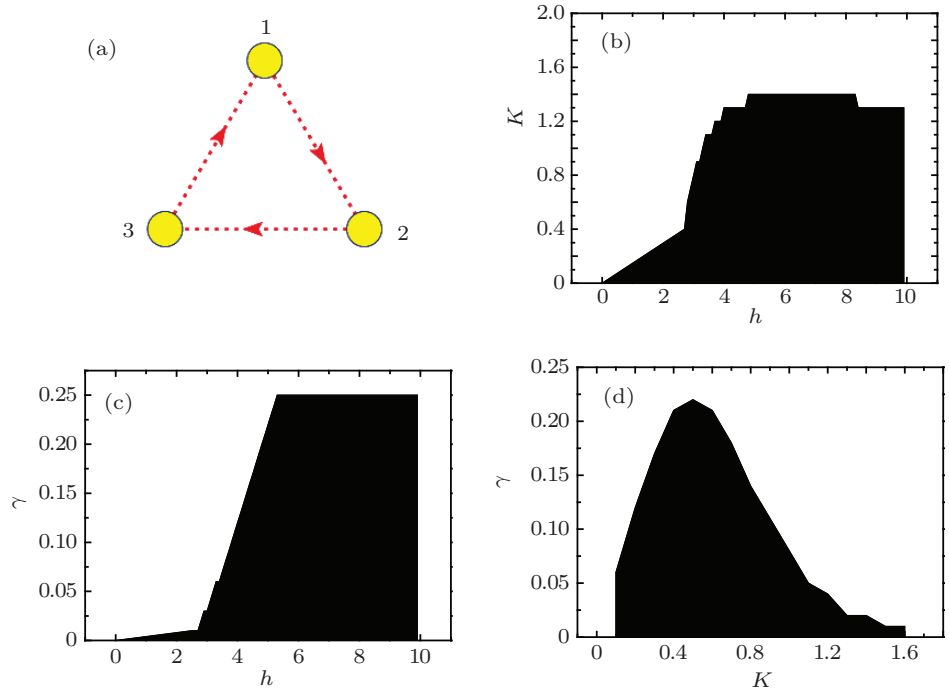


Fig. 1. (colour online) Parameter ranges of oscillation for a repressive 3-node GRN. Panel (a) is the diagram of repressilator network. Green solid lines present activation and red dotted lines repression. Arrows indicate the directions of regulations. (b)–(d) The parameter space of oscillation where oscillation can be found for some initial conditions. In all blank areas, oscillation is not found for arbitrary initial conditions.

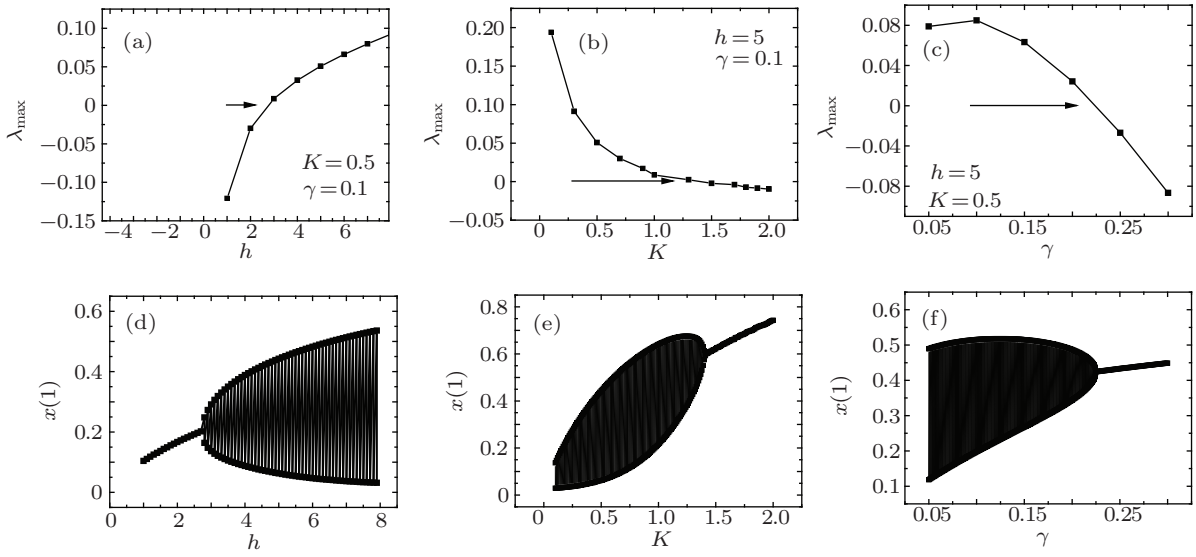


Fig. 2. Hopf bifurcation of network figure: (a) for different parameters; (a)–(c) the maximum real part of the eigenvalues of the GRN plotted versus various parameters. Arrows indicate the parameter values of Hopf bifurcations; (d)–(e) the same as panels (a)–(c), respectively, with the concentrations of $x(1)$ plotted. The behavior of Hopf bifurcation is clearly shown. Before the Hopf bifurcation, solid lines denote the stable steady states, and after the Hopf bifurcation, the two solid lines correspond to the maximum and minimum levels of $x(1)$ during the oscillation.

3.2. Topological characteristics of self-sustaineding oscillatory GRNs

From the tests of a huge number of different topologically distinctive networks, we find that there are some common characteristics in oscillatory GRNs.

We enumerate exhaustively all the 3-node GRNs and 2000 autonomous randomly constructed GRNs 4 and 5-node GRNs, respectively, and compare the numbers of negative loops and positive loops for all oscilla-

tory GRNs. We find that when the number of negative feedback loops ($M_{\rm NL}$) is greater than the number of positive feedback loops ($M_{\rm PN}$) in a GRN, the network has considerably larger probability of generating oscillation than the opposite cases of $M_{\rm PL} > M_{\rm NL}$. Taking 4-node GRNs as examples, in 2000 autonomous randomly constructed GRNs, there are 751 networks with $M_{\rm NL} > M_{\rm PL}$. In these networks, there are 9.99% oscillation generating networks. In comparison, in the networks with $M_{\rm PL} > M_{\rm NL}$, the oscillation probability reduces to 1.81%. In this paper, by oscillatory networks we mean that the networks can generate oscillation at least once for all parameter values and all initial conditions (for each network we conduct 200 random tests). If a network cannot generate any oscillation in all these tests, we call this network non-oscillatory. Table 1 presents the statistical results in detail. All items have the following meanings. n: the number of nodes in a network; TN (test number): the number of randomly constructed networks which meet given conditions; ON (oscillation number): the number of oscillatory networks in TN; R (ratio): the proportion of ON to TN, i.e., R =ON/TN; $M_{\rm NL}$: the number of negative feedback loops in a network; $M_{\rm PL}$: the number of positive feedback loops in a network.

Table 1. Comparison of the number of negative feedback loops $M_{\rm NL}$ and the number of positive feedback loops $M_{\rm PL}$ in GRNs.

n		NN	NP	$M_{\rm NL} > M_{\rm PL}$	$M_{\rm NL} = M_{\rm PL}$	$M_{\rm NL} < M_{\rm PL}$
	TN	1263	1199	1174	774	1336
3	ON	0	34	42	6	0
	R	0	2.84%	3.58%	0.78%	0
	TN	203	184	751	367	882
4	ON	0	7	75	11	16
	R	0	3.80%	9.99%	2.99%	1.81%
	TN	25	25	755	196	1049
5	ON	0	2	78	9	34
	R	0	8.00%	10.33%	4.59%	3.24%

NN indicates the number of networks without negative loop, and NP without positive loop. For 3-node GRNs, we exhaustively enumerated all the 3284 irreducibly distinctive networks, for 4, 5-node GRNs, we randomly construct 2000 autonomous networks in each case.

Table 1 shows that the number of networks which have neither negative or positive feedback loops decreases rapidly as n increases. None of the networks which had no negative feedback loop, without any exception, could generate oscillation, firmly supporting Thomas's conjecture.[19] From Table 1, our further and basic conclusion is that negative feedback loops are in favor of oscillation generation, while positive feedback loops tend to repress oscillation, and the ratio of $M_{\rm NL}$ over $M_{\rm PL}$ is a relevant quantity for network oscillation. It is interesting to observe that there are 1336 3-node networks for $M_{\rm PL} > M_{\rm NL}$ which do have some negative feedback loops, none of which can generate oscillation.

Since all oscillatory GRNs contain at least one negative feedback loop, we call all the irreducible negative feedback loops oscillatory motifs.[23] For self-sustained oscillations of complex GRNs, the existence of one or more oscillatory motifs is a necessary, but not solely sufficient condition. In Fig. 3, all oscillatory motifs of GRNs for $q \leq 5$ are demonstrated. We randomly construct 1000 4-, and 1000 5-node oscillatory GRNs, and find numerically that there are some oscillatory motifs that appear in high-frequency in oscillatory GRNs. Table 2 presents the frequencies of all the oscillatory motifs. We find that in 4-node networks, the first four oscillatory motifs all appear in high-frequency (for a 4-node GRNs, there are only five oscillatory motifs), and in 5-node networks, the first five motifs appear very often in oscillatory GRNs.

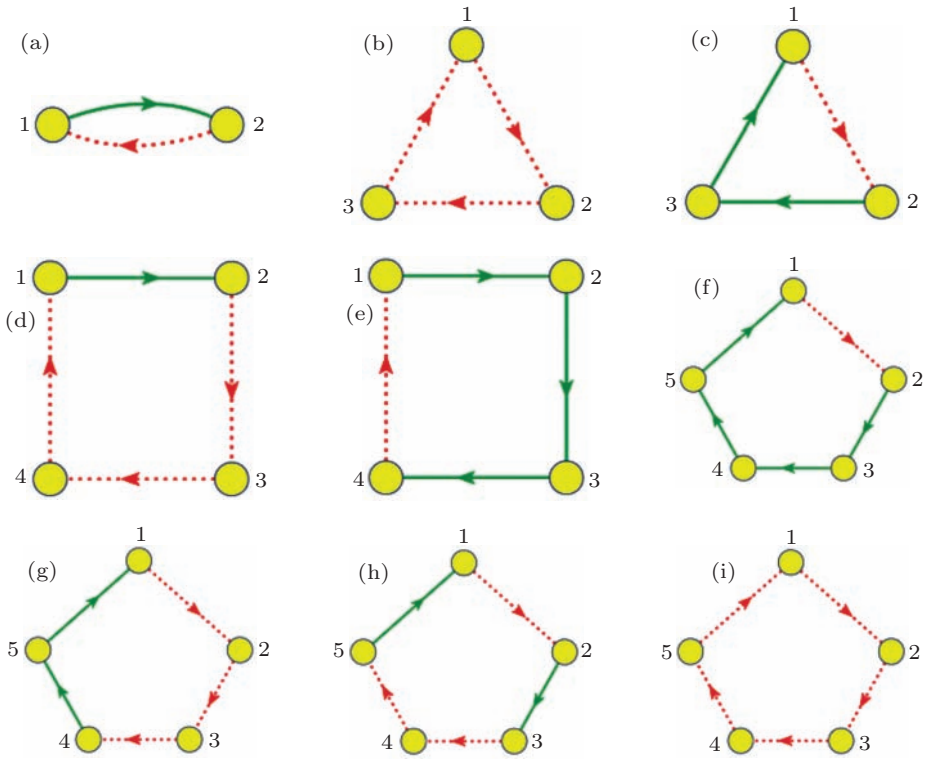


Fig. 3. (colour online) All q-node oscillatory motifs of GRNs for $q \leq 5$: (a) 2-node motif $M(1)$, (b) 3-node motif with three repressive interactions $M(2)$; (c) 3-node motif with a single repressive interaction $M(3)$; (d) 4-node motif with three repressive interactions $M(4)$; (e) 4-node motif with a single repressive interaction $M(5)$; (f) 5-node motif with a single repressive interaction $M(6)$; (g) 5-node motif with three repressive interactions (Type 1) $M(7)$; (h) 5-node motif with three repressive interactions (Type 2) $M(8)$; (i) 5-node motif with five repressive interactions $M(9)$.

Table 2. Frequencies of all the oscillatory motifs shown in Fig. 3 as they appear in 1000 randomly constructed 4- and 5-node GRNs.

Oscillatory motif	1	2	3	4	5	6	7	8	9
4 node	933	673	599	500	90				
5 node	968	779	841	859	506	175	391	448	200

If a motif appears more than once in a given GRN, we count just one time.

3.3. Oscillatory motifs and their efficiencies

In some cases, scientists have been tasked to construct various oscillatory GRNs to achieve specifical biological functions. How to make GRNs oscillatory is a vital issue. It is now well known that a necessary condition for a GRN to exhibit self-sustaining oscillation is that there must exist at least one negative feedback loop, or one oscillatory motif. The different oscillatory motifs in Fig. 3 may have different effects. It is interesting and important to rank the efficiencies of various motifs and to reveal the ones having the strongest effect on oscillation. To our knowledge, these problems have never been considered so far.

We randomly construct 100 million 5-node GRNs, and then pick up the networks which have only one kind of oscillatory motif and classify them. For each class of networks containing only one given motif, we further randomly choose 1000 networks. By numerically simulating these 1000 networks we find the ones that generate sustained oscillation. The numbers of oscillatory networks may represent the effects of the corresponding oscillatory motifs, because every GRN studied has only a single type of oscillatory motif. It is found that there are some motifs which have much higher probabilities than others for generating oscillation. Consider motif $1(M(1))$ which appears with extremely high-probability in most of GRNs (see Table 2). We then consider networks having two types of motifs (one of which is $M(1)$), to study the combinative effects of the two motifs to oscillation. Table 3

presents also these statistical results in the 3rd and 4th lines.

The notation in Table 3 has the following meaning: Only(K) is the ratio of the number of GRNs which have only $M(K)$ to the total number of GRNs randomly chosen; Only(OK) represents the ratio of the number of oscillatory GRNs having only $M(K)$ to the total number of GRNs with the same type of oscillatory motifs; Only$(1, K)$ is the ratio of the number of GRNs having two types of $M(1)$ and $M(K)$ to the total number of GRNs randomly chosen; Only(O$(1, K)$) represents the ratio of the number of oscillatory GRNs having $M(1)$ and $M(K)$ to the number of GRNs having the same types of motifs. From Table 3, two important conclusions can be extracted. First, $M(2)$ is the most effective for generating oscillation. Generally speaking, the efficiency of motifs decline as the nodes of the oscillatory motifs increase. The efficiencies of different motifs can be approximately ranked as $2 > 1 > 3 > 4 > 9 > 5, 6, 7, 8$, where the effects of $M(6)$–$M(8)$ can be neglected (the products of (K) multiplied by Only(OK) and Only$(1, K)$ multiplied by Only(O$(1, K)$) are very small). Second, networks with combinative actions of $M(1)$ and $M(K)$ can greatly enhance the efficiency of the $M(K)$. For example, the joint effects of $M(1, 2)$ can greatly increase the probability of oscillation Only$((1, 2)$ multiplied by Only(O$(1, 2)) \gg (2)$ multiplied by Only(O(2))), and this probability is also considerably larger than all other possible productions.

Table 3. Efficiencies of different oscillatory motifs for generating oscillation.

Motif(K)	1	2	3	4	5	6	7	8	9
Only(K)	2.49%	0.21%	0.72%	0.11%	0.11%	0.008%	0.0078%	0.008%	0.002%
Only(OK)	2.37%	5.13%	1.33%	0.87%	0.07%	0	0.03%	0.07%	0.5%
Only$(1, K)$	2.49%	1.06%	3.92%	0.82%	0.82%	0.072%	0.07%	0.068%	0.013%
Only(O$(1, K)$)	2.37%	10.70%	2.90%	3.80%	1.30%	0.70%	4.20%	6.20%	7.40%

In Ref. [23] we studied the problem of oscillation sources in GRN systems by using dominant phase-advanced driving (DPAD) method. We find all oscillation sources are negative feedback loops (i.e., oscillatory motifs here). In a GRN there are usually many oscillatory motifs, only one or a few motifs can actually serve as the oscillation sources in the given oscillatory GRN. Hence, there is a new problem in which motifs that have large probabilities serve as source loops in arbitrary GRNs. For 3-node GRNs we exhaustively enumerate all the 3284 3-node GRNs; for 4- and 5-node GRNs, we randomly construct 2000 autonomous networks for each. By applying the DPAD method,[24−26] we can define the oscillation generator for each actual oscillatory GRN. The probabilities of different oscillatory motifs acting as oscillation generators are shown in Table 4. In all 3-node GRNs, there are only 48 networks generating sustained oscillation, in which there are two oscillatory motifs (i.e., $M(1)$ and $M(2)$) acting as the oscillation generator, with $M(2)$ being the most frequent of the two. It is interesting to notice that though the $M(3)$ does exist in 3-node GRNs, it never plays the role of oscillation generator there. For 2000 4-node GRNs, there are 102 networks generating sustained oscillation. In all these oscillatory realizations, two ($M(2)$ and $M(3)$) oscillatory motifs act as the oscillation sources, and the probability of the oscillation generator being M(2) is much higher than that of the $M(3)$. For 2000 5-node GRNs, there are 121 networks generating oscillation. Now, there are four oscillatory motifs (i.e., $M(2)$, $M(3)$, $M(4)$, and $M(5)$) acting as oscillation generators. Three conclusions are clear in Table 4. First, $M(2)$ has the highest probability, much higher than all other motifs, to act as oscillation generator. Therefore, this motif plays a very important role on network oscillation. This is unanimous with the conclusion of Table 3. Meanwhile, this explains why the repressilator figure 1(a) has been studied so extensively[27,28] and constructed foremost to achieve oscillation.[14] Second, not all oscillatory motifs actually play the role of oscillation source. For instance, $M(3)$ in 3-node GRNs, $M(1)$ in 4- and 5-node GRNs have extremely low probabilities (if there are any not caught in our extensive tests) acting as the oscillation generators. Nevertheless, these motifs may still play role in yielding oscillatory behaviors, cooperating with other motifs.

For 3-node GRNs, we exhaustively enumerate all the 3284 networks, and for 4- and 5-node GRNs, we randomly construct 4000 (2000 for each) autonomous networks in total. N is the frequency of each oscillatory motif acting as the oscillatory generator. R represents the ratio of N over the total oscillation number.

Table 4. The probability for an oscillatory motif acting as an oscillation generator (i.e., the source loop defined in DPAD patterns[23,26]).

K		Oscillation number	1	2	3	4	5	6	7	8	9
3	N	48	22	26							
	R		45.83%	54.17%							
4	N	102		91	11						
	R			89.22%	10.78%						
5	N	121		64	39	15	3				
	R			52.89%	32.23%	12.40%	2.48%				

Tables 3 and 4 show that the proportion of negative interactions in an oscillatory motif is also a significant index to estimate the efficiency of the oscillatory motif. This efficiency rises as the proportion increases. For example, comparing $M(2)$ and $M(3)$, we find that in $M(2)$ all interactions are repressive while only 33.3% are repressive in $M(3)$. The ratio of oscillatory GRNs is 5.13% for $M(2)$ while it is 1.33% for $M(3)$. The conclusion is also valid in 4- and 5-node oscillatory motifs.

3.4. Anti-oscillatory motifs (AM) and their efficiencies

In Subsection 3.3, we find that there are some effective oscillatory motifs generating oscillation in GRNs. Nevertheless, we observe also many GRNs, which have these effective oscillatory motifs but which do not produce sustained oscillation. The reason may be that in GRNs there are some other motifs which repress oscillation. Goh conjectured that a network generating oscillation is the result of the competition between positive feedback loops and negative feedback loops.[21] In Fig. 4, all the positive feedback loops of GRNs for $q \leq 5$ are presented, and we term them anti-oscillatory motifs. In Table 5, similar to Table 3, we investigated the negative effects of all the anti-oscillatory motifs in repressing oscillation. We find again that there are some anti-oscillatory motifs having very strong oscillation repressing effects and some others have rather weak effects. The notations Only(K) and Only(OK) have the same meanings as those in Table 3. From the results, we observe that $AM(12)$ has the lowest oscillation repressing effect, while $AM(3)$ and $AM(11)$ have the strongest.

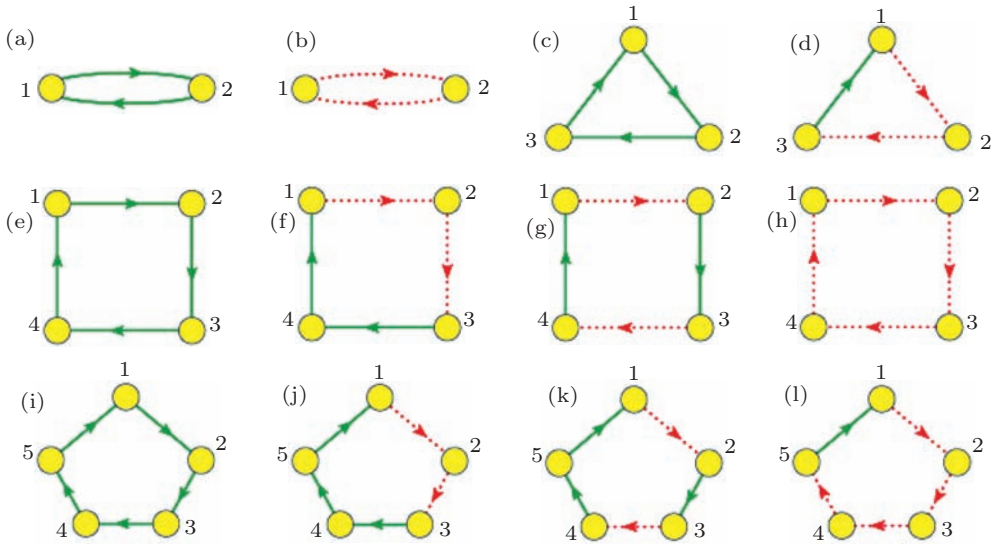


Fig. 4. (colour online) All anti-oscillatory q-node motifs (positive feedback loops) of GRNs for $q \leq 5$: (a) 2-node motif with two active interactions $AM(1)$; (b) 2-node motif with two repressive interactions $AM(2)$; (c) 3-node motif with three active interactions $AM(3)$; (d) 3-node motif with two repressive interactions $AM(4)$; (e) 4-node motif with four active interactions $AM(5)$; (f) 4-node motif with two successive repressive interactions $AM(6)$; (g) 4-node motif with two separate repressive interactions $AM(7)$; (h) 4-node motif with four repressive interactions $AM(8)$; (i) 5-node motif with five active interactions $AM(9)$; (j) 5-node motif with two successive repressive interactions $AM(10)$; (k) 5-node motif with two separate repressive interactions $AM(11)$; (l) 5-node motif with four repressive interactions $AM(12)$.

Table 5. Effects of different anti-oscillatory motifs to the oscillation suppression.

Motif(K)	1	2	3	4	5	6
Only(K)	1.01%	1.02%	0.22%	0.81%	0.035%	0.072%
Only(OK)	5.40%	8.80%	2.10%	9.50%	8.40%	13.40%
Motif(K)	7	8	9	10	11	12
Only(K)	0.15%	0.036%	0.002%	0.013%	0.014%	0.014%
Only(OK)	4.20%	31.30%	18.67%	20.60%	1.60%	32.00%

Comparing Fig. 4 and Table 5, it is also found that the effects of anti-oscillatory motifs are related to the number and distribution of negative interactions. For q-node anti-oscillatory motifs the greater the number of negative interactions, the lower the oscillation repressing effect (Figs. 4(h) and 4(l)). However, if the negative feedbacks are not successively linked (Figs. 4(g) and 4(k)), the repressing effect to oscillation of the motifs can rise considerably. Taking the four 4-node anti-oscillatory motifs figures 4(e)–4(h) as examples, $AM(8)$ has four negative feedback, it therefore has the lowest oscillation repressing effect; $AM(6)$ has two successive negative interactions, the effect becomes slightly stronger; $AM(7)$ has also two negative interactions, but the negative interactions are detached, so it has the strongest oscillation repressing effect. All the above conclusions are gained from the summary of the statistics in extensive tests. The analytic proofs are not available, and the mechanisms underlying these conclusions are still under investigation.

4. Discussion and conclusion

Self-sustained oscillatory GRNs are important for various living organisms. Oscillation appears only within proper parameter ranges. By numerical simulation and investigation on statistics with a large amount of computation data, we find that the parameters of small γ, large h, and certain optimal K favor a high probability of oscillation. This finding is useful for practical oscillation generation by adjusting biological control parameters.

This paper attempts to investigate the characteristic features of the oscillatory GRNs. From numerical statistics, it is found that in most oscillatory GRNs, the number of negative feedback loops is no less than the number of the positive feedback loops. This result supports the conclusion by Goh who suggested that whether or not a network could generate oscillation depends on the result of the competition between positive and negative feedback loops.[21] Furthermore, the relation between the oscillation probability and oscillatory motifs is also studied. Numerical statistics shows that there are some very effective oscillatory motifs, in particular, the 3-node loop of successively repressive interactions, playing central role in generating persistent oscillation (Tables 3 and 4). On the other hand, there also exist anti-oscillatory motifs (positive feedback loops) which play a role in repressing oscillation. Some anti-oscillatory motifs have a much stronger effect in repressing oscillation than other motifs (Table 5). It is well known that positive (negative) feedback loops play an important role in GRNs. We therefore consider only the directed ring structures embedded. Of course, there are some other kinds of structures for the regulatory mechanism. However, the task of the present research focuses on the influences of positive (negative) feedback loops, and leaves other facts to possible future works.

With these conclusions, scientists may increase the probability of oscillatory GRNs (in case of need) through various ways. Firstly, we can increase the number of the negative feedback loops, or decrease the number of positive feedback loops. Secondly, we may try to increase some of the most effective oscillatory motifs shown in Tables 3 and 4. Third, we can also reduce the strongest anti-oscillatory motifs denoted in Table 5. All these methods may help up to increase the probability of oscillation in practical biological GRNs.

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