2004 International Symposium on Nonlinear
Theory and its Applications (NOLTA2004)
Fukuoka, Japan, Nov. 29 - Dec. 3, 2004

Designing Synthetic Gene Oscillators by Negative Feedback Networks

Ruiqi Wang, Tianshou Zhou, Luonan Chen
Osaka Sangyo University, Nakagaito 3-1-1, Daito, Osaka 574-8530, Japan. Email: chen@elec.osaka-sandai.ac.jp

Abstract- **This paper focuses on developing a new methodology to model and design periodic oscillators of gene regulatory networks with multiple genes, proteins and time delays, by using multiple time-scale networks (MTN). Multiple time scale properties are exploited to simplify the model according to singular perturbation theory. We show that a MTN has no stable equilibria but stable periodic orbits. Finally, a biologically plausible gene oscillator is designed to demonstrate the theoretical results.**

1 Introduction

Rhythmic phenomena exist at all levels among living organisms with periods ranging from less than a second to years [1, 2, 3]. From both theoretical and experiment viewpoints, it is a great challenging problem in biological science to model, analyze and further predict the periodic behaviors of bio-systems. On the other hand, recent progress in genetic engineering has made the design and implementation of artificial or synthetic gene networks realistic from both theoretical and experimental viewpoints [5], in particular for simple organisms, such as *E.coli* and *yeast* [1, 4].

Explicitly considering all variables and chemical reactions in a cell is unrealistic for a gene regulatory network from modelling, analyzing and computing viewpoint. However, in a cell, many different time scales characterize the gene regulatory processes, which can be exploited to reduce the complexity of the mathematical models [1, 4]. For instance, the transcription and translation processes in the gene network generally evolve on a time scale that is much slower than that of phosphorylation, dimerization or binding reactions of transcription factors in the protein network. Such properties can be also exploited to simplify the model provided that the simplified system is guaranteed to behave both qualitatively and quantitatively as the original one.

This paper aims to develop a new methodology to analyze and design biological oscillating networks with time delays, by using multiple time-scale networks (MTN). We show that a MTN with certain conditions has no stable equilibria but stable periodic oscillations, depending on the total time delay, although it has a complicated network structure including both positive and negative feedback loops. As an implementation example, a biologically plausible two-gene synthetic model with genes *lac* and *cI* is designed to demonstrate the theoretical result.

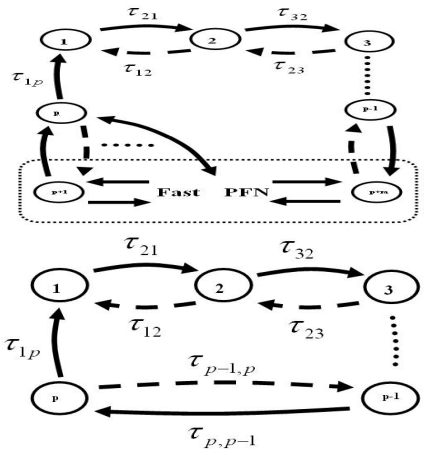

Figure 1: An illustration of MTN. (a): An example of a basic MTN. (b): The reduced MTN of (a).

2 MTN

Generally, the dynamics of a gene network primarily including the gene regulatory reactions, such as the transcription and translation processes, evolves on a time scale that is much slower than those of a protein network mainly including protein reactions, such as phosphorylation, dimerization or binding reactions. In addition, although dynamics are intertwined between gene network and protein network or metabolic network, topological structure of interactions for each network is relatively independent of each other. A MTN [8] is constituted by exploiting such properties to transform a complicated biological model into a simplified but dynamically equivalent system.

2.1 Basic MTN

Assume the system in this paper to be a monotone dynamical system, i.e. each element of its Jacobian matrix J has the fixed sign for all $x \in X$. A edge between nodes i and j is defined as a Jacobian element J_{ij}. A loop is negative (or positive) if the product of its edges connecting the loop is negative (or positive) for all $x \in X$. A basic MTN consists of a fast positive feedback network (PFN) [5, 8] and a slow cyclic feedback network (CFN) [8]. Assume that there are m fast variables $y = (y_1, \cdots, y_m)$ and p slow variables $x = (x_1, \cdots, x_p)$, representing the concentrations of chemical components at time t, where $p \geq 2$. As shown in (a) of figure 1, then a MTN [8] can be written

as

$$\begin{aligned}
\dot{x}_1(t) = &\quad f_1(x_2(t-\tau_{12}), x_1(t), x_n(t-\tau_{1,n})) \\
\dot{x}_i(t) = &\quad f_i(x_{i+1}(t-\tau_{i,i+1}), x_i(t), x_{i-1}(t-\tau_{i,i-1}), y_t) \\
&\quad 2 \leqslant i \leqslant p-2 \\
\dot{x}_{p-1}(t) = &\quad f_{p-1}(y_t, x_{p-1}(t), x_{p-2}(t-\tau_{p-1,p-2})) \\
\dot{x}_p(t) = &\quad f_p(x_p(t), y_t), \qquad (1) \\
\epsilon\dot{y} = &\quad g(x_{p-1}(t-\tau_{p-1}), x_p(t-\tau_p), y_t) \qquad (2)
\end{aligned}$$

where $y_t \equiv y(t+\theta)$, $-r \leq \theta \leq 0$. $\tau_{i+1,i} = \tau_{i,i+1} = 0$ for $1 \leq i \leq p-1$ if both $\partial f_{i+1}/\partial x_i$ and $\partial f_i/\partial x_{i+1}$ are nonzero, and $\tau_{i+1,i}$ maybe any non-negative finite real number if $\partial f_i/\partial x_{i+1} = 0$. ϵ is a small parameter (> 0). All loops in eqn.(2) of PFN are positive and

$$\frac{\partial f_i(\eta,\xi,\zeta)}{\partial \eta}\frac{\partial f_{i+1}(\eta,\xi,\zeta)}{\partial \zeta} \geq 0, \qquad (3)$$

where $\partial f_{i+1}(\eta,\xi,\zeta)/\partial\zeta \neq 0$, for $1 \leq i \leq p-1$. Assume that eqns.(1)-(2) are bounded due to biological restriction.

Eqns.(1)-(2) are called a *singularly perturbed system* also known as a fast-slow system with slow x and fast y. Such multiple time-scale properties are found in many biochemical systems, in particular gene regulatory systems [1, 4]. Fig.1(a) is an example of MTN. Slow subnetwork is composed of p slow chemical components from the 1st node to the p-th node. Note that there is at least one single-direction interaction in the slow subnetwork, i.e., $\partial f_p/\partial x_1 = 0$ in Fig.1(a). Actually, it is not necessary from the p-th to 1-th nodes. Fast subnetwork is comprised of m fast chemical components from the $(p+1)$-th node to the $(p+m)$-th node, and is a PFN. Note that there may be many variables in y interacting with x but only two variables in x affecting y.

When $\epsilon = 0$, due to the properties of monotone dynamical system [7], as shown in (b) of figure 1, eqns.(1)-(2) degenerate to a CFN, i.e.,

$$\begin{aligned}
\dot{x}_1(t) = &\quad \hat{f}_1(x_2(t-\tau_{12}), x_1(t), x_n(t-\tau_{1,n})) \\
\dot{x}_i(t) = &\quad \hat{f}_i(x_{i+1}(t-\tau_{i,i+1}), x_i(t), x_{i-1}(t-\tau_{i,i-1})) \\
\dot{x}_p(t) = &\quad \hat{f}_p(x_p(t), x_{p-1}(t-\tau_{p,p-1})), 2 \leqslant i \leqslant p-1 \qquad (4)
\end{aligned}$$

This system is called *reduced system*. We can prove the following theorem.

Theorem 1 An orbitally and asymptotical stable periodic-solution $x = \Phi(t)$ of eqn.(4) is stable under persistent perturbations. Moreover, for sufficiently small ϵ, $x = \Phi(t)$ is a stable periodic solution of eqns.(1)-(2).

Based on the monotone dynamical system theory and discrete Lyapunov functional [6], We can show that the Poincaré-Bendixson type Theorem holds [8] for the monotone CFN eqn.(4).

Theorem 2 *Let $x(t)$ be a solution of eqn.(4) on some time interval $[t^0, \infty)$, and $\hat{f}$ satisfy eqn.(3). Then either*

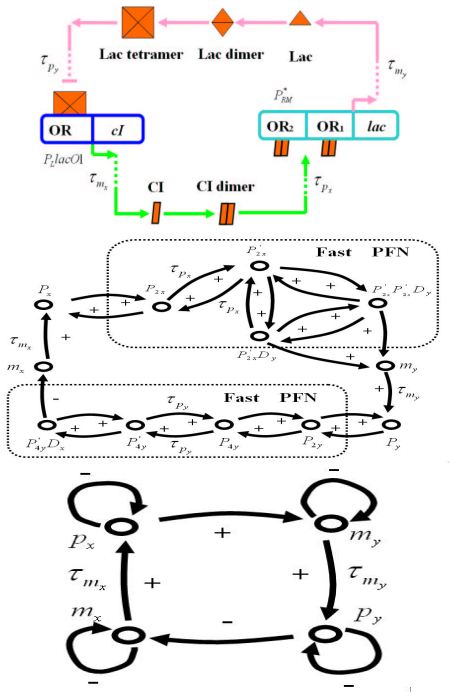

Figure 2: An implementation. (a): Schematic for the synthetic gene network by *cI* and *Lac* genes. (b): Schematic of MTN for the synthetic gene regulatory network shown in (a). (c): The reduced MTN.

1. *$\omega(z)$ is a single non-constant periodic orbit; or else*
2. *for each solution $u(t)$ of eqn.(4) in $\omega(x)$, i.e., for solutions with $u_t \in \omega(x)$ for all $t \in \mathbb{R}$, we have*

$$\alpha(u) \cup \omega(u) \subseteq E, \qquad (5)$$

where $\alpha(u)$ and $\omega(u)$ denote the alpha- and omega-limit sets, respectively, of this solution, and where E denotes the set of equilibria of eqn.(4).

A PFN is robust to time delay variations, whereas time delays in CFN may significantly affect the dynamics of network. As indicated in eqn.(4), different from the time delays in the slow subnetwork, the time delays in the fast subnetwork or PFN has no effect on the asymptotical dynamics of the original MTN or the reduced MTN. In other words, we do not need care about the time delays in the fast subsystems for analyzing or designing gene oscillators, although they may influence the system for the transient dynamics.

2.2 Generalized MTN

The result for the basic MTN with one fast PFN can be easily extended to a general MTN with multiple fast PFNs. Provided that each PFN interacts with two neighboring variables in x_t, Theorems 1 and 2 still hold for the corresponding CFN or the reduced MTN. Although Poincaré-Bendixson type Theorem shows that omega-limit sets of CFNs are composed of

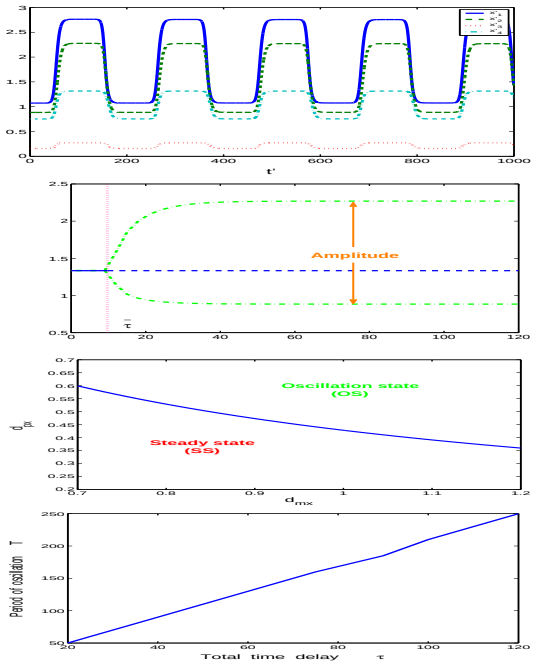

Figure 3: Results. (a): Sustained oscillations by the MTN at $d_{px} = 0.5$ min^{-1}, $d_{mx} = 1$ min^{-1} and $\tau = 100$. (b): A bifurcation diagram with total time delay τ as a parameter at $d_{px} = 0.5$ min^{-1} and $d_{mx} = 1$ min^{-1}. (c) :The oscillatory region (OS) and steady state region (SS) at $\tau = 100$. (d): Total delay τ and period T.

only periodic orbits and equilibria, it does not provide sufficient conditions for periodic orbits. The sufficient conditions and detailed proofs of these results for periodic orbits of the reduced MTNs can be found in [8].

3 Numerical implementation

In this section, we demonstrate our theoretical results by designing a synthetic gene network, which is actually a MTN and consists of two fast PFNs and one slow CFN. As shown in Fig.2(a), the synthetic gene regulatory network is a simple two-gene model with genes *cI* and *lac* under the control of promoters $P_L lacO1$ and P^*_{RM} respectively. All two genes are both well-characterized transcriptional regulators, which can be found in bacterium *E.coli* and λ phage. We assume that the designed gene network is implemented in a eukaryotic cell, e.g. in *yeast*, so as to examine the effect of time delays on the oscillation dynamics. mRNA or m_x of gene *cI* translates the protein CI or p_x in cytoplasm, which in turn forms a homodimer p_{2x} and is transported or diffused into the nucleus in the form p'_{2x} to enhance the expression of gene *Lac* by binding on the two operator sites of the promoter P^*_{RM}. On the other hand, mRNA or m_y of gene *lac* translates the protein Lac or p_y, which forms a homodimer p_{2y} and further a tetramer p_{4y} in the cytoplasm. When moved to the nucleus, the tetramer p_{4y} is in the form of p'_{4y}, which represses the expression of gene *cI* by binding on the operator site of the promoter $P_L lacO1$. The promoters $P_L lacO1$ has one binding site OR for Lac tetramer, but the promoter P^*_{RM} has two binding sites OR_1 and OR_2 for CI dimers with the affinity priority binding first on OR_1 and second on OR_2. Note that P^*_{RM} is a mutated promoter from P_{RM}, which has no binding site for the tetramer Lac. The Schematic for the synthetic gene network is shown in Fig.2(a). Different from prokaryotes, there are time delays $(\tau_{mx}, \tau_{my}, \tau_{px}, \tau_{py})$ due to transportation or diffusion of mRNAs and transcriptional factors between the nucleus and cytoplasm, which may significantly affect the dynamics of the system. Such a circuit can be engineered on plasmids, and then be cloned to multiple copies, e.g., by PCR. The engineered plasmids are further assumed to grow in *yeast*, by injecting into *yeast* and recombining into their genome.

As shown in (b) of figure 2, we define the following chemical species in terms of concentrations: m_x, mRNA CI; p_x, CI protein; p_{2x}, CI dimer in cytoplasm; p'_{2x}, CI dimer in nucleus; D_y, the free DNA binding or operator site in promoter P^*_{RM}; $p'_{2x}D_y$, CI dimer bound to operator site OR_1 of promoter P^*_{RM}; $p'_{2x}p'_{2x}D_y$, CI dimers bound to both OR_1 and OR_2 of promoter P^*_{RM}; m_y, mRNA Lac; p_y, Lac protein; p_{2y}, Lac dimer; p_{4y}, Lac tetramer in cytoplasm; p'_{4y}, Lac tetramer in nucleus; D_x, the free DNA binding site in promoter $P_L lacO1$; $p'_{4y}D_x$, Lac tetramer bound to the operator site OR of promoter $P_L lacO1$. The fast reactions are mainly multimerization and binding reactions for protein network. As indicated in figure 2(b). On the other hand, the slow reactions involve transcription of mRNAs and translation of proteins, and degradation of proteins and mRNAs. There are also conservation conditions for total binding sites of promoters, i.e., $D_y + p'_{2x}D_y + p'_{2x}p'_{2x}D_y = n_y$ and $D_x + p'_{4y}D_x = n_x$, where n_x and n_y are the concentration of genes *cI* and *lac*, respectively. For convenience, m_x is denoted by X_1, p_x by X_2, m_y by X_3, p_y by X_4, p_{2x} by Y_1, p'_{2x} by Y_2, $p'_{2x}D_y$ by Y_3, $p'_{2x}p'_{2x}D_y$ by Y_4, p_{2y} by Y_5, p_{4y} by Y_6, p'_{4y} by Y_7 and $p'_{4y}D_x$ by Y_8. Then we have

$$\begin{aligned}
\frac{dX_1}{dt} &= k_{mx0}(n_x - Y_8) + k_{mx1}Y_8 - d_{mx}X_1 \\
\frac{dX_2}{dt} &= k_{px}X_1(t-\tau_{m_x}) + 2k_{-1}Y_1 - 2k_1X_2^2 - d_{px}X_2 \\
\frac{dX_3}{dt} &= k_{my0}(n_y - Y_3 - Y_4) + k_{my1}Y_3 \\
&\quad + k_{my2}Y_4 - d_{my}X_3 \\
\frac{dX_4}{dt} &= k_{py}X_3(t-\tau_{m_y}) - 2k_5X_4^2 + 2k_{-5}Y_5 - d_{py}X_4
\end{aligned}$$

$$\begin{aligned}
\frac{dY_1}{dt} &= k_1X_2^2 + k_{-2}Y_2(t-\tau_{p_x}) - k_{-1}Y_1 - k_2Y_1 \\
\frac{dY_2}{dt} &= k_2Y_1(t-\tau_{p_x}) - k_{-2}Y_2 + k_{-3}Y_3 \\
&\quad - k_3(n_y - Y_3 - Y_4)Y_2 + k_{-4}Y_4 - k_4Y_2Y_3 \\
\frac{dY_3}{dt} &= k_3(n_y - Y_3 - Y_4)Y_2 + k_{-4}Y_4 \\
&\quad - k_4Y_2Y_3 - k_{-3}Y_3
\end{aligned}$$

$$
\begin{aligned}
\frac{dY_4}{dt} &= k_4 Y_2 Y_3 - k_{-4} Y_4 \\
\frac{dY_5}{dt} &= k_5 X_4^2 - k_{-5} Y_5 - 2k_6 Y_5^2 + 2k_{-6} Y_6 \\
\frac{dY_6}{dt} &= k_6 Y_5^2 - k_{-6} Y_6 + k_{-7} Y_7(t-\tau_{p_y}) - k_7 Y_6 \\
\frac{dY_7}{dt} &= k_7 Y_6(t-\tau_{p_y}) - k_{-7} Y_7 \\
&\quad -k_8 Y_7(n_x - Y_8) + k_{-8} Y_8 \\
\frac{dY_8}{dt} &= k_8(n_x - Y_8) Y_7 - k_{-8} Y_8. \qquad (6)
\end{aligned}
$$

Parameters are mainly from [4] with slight modifications, and are set as $k_{mx1} = 0.2$ min^{-1}, $K_8 = 2\times10^{13}$ M^{-1}, $n_x = 1$ nM, $n_y = 1$ nM, $K_6 = 10^7$ M^{-1}, $K_5 = 10^8$ M^{-1}, $k_{mx0} = 3$ min^{-1}, $k_{px} = 4$ min^{-1}, $k_{my1} = 3$ min^{-1}, $k_{my2} = 12$ min^{-1}, $K_1 = 5\times10^7$ M^{-1}, $K_3 = 3\times10^8$ M^{-1}, $d_{my} = 5$ min^{-1}, $k_{my0} = 2$ min^{-1}, $k_{py} = 1$ min^{-1}, $d_{py} = 2$ min^{-1} and $\sigma = 2$. Other parameters are given when they are used. According to the above parameters, the variables are scaled as X_1 (nM) $\sim 0.8x_1$, X_2 (nM) $\sim 8x_2$, X_3 (nM) $\sim 8x_3$, $X_4 \sim 0.8x_4$ and t (min) $\sim t'/1.37$. Note that τ is also a scaled time delay by 1.37. As shown in (c) of figure 2, the MTN is reduced to a CFN according to Theorems 1 and 2.

Fig.3(a) shows a case for sustained oscillations generated with $d_{px} = 0.5$ min^{-1}, $d_{mx} = 1$ min^{-1} and $\tau = 100$, which confirms our theoretical prediction. Because the fast reactions as perturbations do not change their period or amplitude in the long run, limit cycle oscillations represent a particularly stable mode of periodic behavior. Such stability holds with the robust nature of circadian clocks which have to maintain their amplitude and period in changing environment.

The bifurcation diagram for x_2 is shown in Fig.3(b), where the control parameter is the total time delay τ. The solid line and dashed lines represent stable or unstable equilibrium, respectively. The dash-dotted lines indicate the maximum and minimum values of x_2' for the sustained oscillation. Limit cycles exist when $\tau > \bar{\tau}$ for which the equilibrium is unstable. At low values of τ, the system reaches a stable steady state corresponding to some constant concentrations of the state variables. With the increase of τ, a bifurcation occurs at a critical value $\bar{\tau} = 10.65$. After $\tau > \bar{\tau}$, the steady state becomes unstable and sustained oscillations occur. The amplitudes of the sustained oscillations are also shown in Fig.3(b). When $\tau = \bar{\tau}$, we get a pair of imaginary roots $\lambda = \pm 0.20j$ for the characteristic equation, which corresponds to a Hopf bifurcation point. Moreover, from Fig.3(b), the amplitudes increase with the time delay τ when τ is small, which means that the time delay can be used to control the amplitudes. However, when τ is large, the amplitudes is not sensitive to τ.

The oscillatory region (OS) and steady state region (SS) at $\tau = 100$ are shown in Fig.3(c), from which we can see that oscillations are generally enhanced with the increase of the degradation rates of mRNA *cI*.

The analysis of the effect for total time delay τ on the period of oscillation T is Fig.3(d). In addition to amplitudes as shown in Fig.3(b), the period of oscillation, namely T, increases with the total time delay τ in almost a linear way. Therefore, the total time delay τ can be viewed as a key parameter to control both amplitude and period of an oscillation in a biological system.

There are mainly four delays, (τ_{mx}, τ_{my}) and (τ_{px}, τ_{py}) representing transportation or diffusion processes from nucleus to cytoplasm of mRNAs and from cytoplasm to nucleus of proteins respectively, which play different roles in dynamical behaviors of the system. Since τ_{px} and τ_{py} are in PFNs, they have no effects on the asymptotical dynamics. On the other hand, τ_{mx} and τ_{my} both qualitatively and quantatively affect the dynamcial behaviors not separately but in the form of $\tau = \tau_{mx} + \tau_{my}$, due to cyclic structure of the CFN.

4 Conclusion

We developed a new methodology to analyze and design biological oscillating networks with time delays, by using MTNs. We first describe a basic MTN with only one PFN, and then extended our result for multiple PFNs, which enables our model to apply to wider systems for modelling and designing bio-oscillators. As indicated in this paper, in contrast to the time delays in the slow subnetwork that significantly affect the dynamics of the system, the time delays in the fast subnetwork or PFN has no effect on the asymptotical dynamics of the MTN although they may play an important role in the transient dynamics. Such a property is important in designing or modelling gene oscillators when time delays are concerned.

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