A Model of Periodic Oscillation for Genetic Regulatory Systems

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Abstract—In this paper, we focus on modeling and explaining periodic oscillations in gene–protein systems with a simple non-linear model and on analyzing effects of time delay on the stability of oscillations. Our main model of genetic regulation comprises of a two-gene system with an autoregulatory feedback loop. We exploit multiple time scales and hysteretic properties of the model to construct periodic oscillations with jumping dynamics and analyze the possible mechanism according to the singular perturbation theory. As shown in this paper, periodic oscillations are mainly generated by nonlinearly negative and positive feedback loops in gene regulatory systems, whereas the jumping dynamics is generally caused by time scale differences among biochemical reactions. This simple model may actually act as a genetic oscillator or switch in gene-protein networks because the dynamics is robust for parameter perturbations or environment variations. We also explore effects of time delay on the stability of the dynamics, showing that the time delay generally increases the stability region of the oscillations, thereby making the oscillations robust to parameter changes. Two examples are also provided to numerically demonstrate our theoretical results.

Index Terms—Bifurcation, circadian rhythm, genetic regulatory system, periodic oscillation, relaxation oscillator, time delay.

I. INTRODUCTION

NONLINEAR phenomena such as multistability, oscillations, and switching exist at various levels of biological processes and organizations [1]–[3] and have been investigated on the basis of many theoretical models, such as circadian oscillations with the period protein (PER) and the timeless protein (TIM) in *Drosophila* [4], [5], and multistable dynamics regulated by transcriptional factors [6]–[8]. Considerable experimental evidence suggests that cellular processes are intrinsically rhythmic or periodic [1]. Various periodic oscillations with different time scales ranging from less than a second to more than a year, which may allow for living organizms to adapt their behaviors to a periodically varying environment [3], [5], have also been observed experimentally. Moreover, multiple phosphorylation, multimerization, and positive or negative feedback regulation of transcription factors are likely to play important roles

in many sustained oscillations and switching dynamics [4], [7] of biochemical systems.

Mathematical models defined as dynamical systems have been studied extensively for biochemical oscillations and gene expression multistability [8], [9]. For instance, Goodwin proposed an oscillatory reaction scheme to describe the putative molecular mechanism of biological oscillation [1], [10]. Goldbeter modeled the bisphosphorylation of PER and negative feedback regulation of PER with a five-variable dynamical system that numerically elucidated circadian rhythms in *Drosophila* [4]. Smolen, Baxter and Byrne explained the bistability or switching phenomena in gene expression by extending Keller's work [6] and by modeling mainly transcription processes with ordinary differential equations [7], [8]. In synthetic gene networks, utilizing hysteresis-based oscillations has been proposed [11] recently. In particular, Hasty *et al.* showed numerically that a genetic oscillator can be constructed by utilizing hysteretic property of the λ phage circuitry with repressor as a function of time [12], [13]. All of these works stress the importance of feedback regulation of transcriptional factors, which is a key in giving rise to oscillatory or multistable dynamical behaviors exhibited by both natural biological systems and synthetic genetic systems. In addition, it should be noted that many periodic behaviors do not simply oscillate smoothly; rather, they change rapidly or jump at certain states [2], [13]–[15].

In gene expression systems, many different time scales characterize the gene regulatory processes. For instance, the transcription and translation processes generally evolve on a time scale that is much slower than that of phosphorylation, dimerization or binding reactions of transcription factors. In gene–protein networks, the time scale for expression of some genes is much slower than that of others, depending on the length of the genes. In this paper, we aim to explain the robust mechanism of oscillations in biochemical gene-protein systems by a simple nonlinear model and to reveal the existence of a limit cycle with jumping behaviors or a relaxation oscillation by exploiting multiple time-scale properties. This kind of limit cycles have also been observed in neuronal systems [16], [17] and other biological systems. We show that periodic oscillations are mainly generated by nonlinear feedback loops in gene regulatory systems and the jumping dynamics caused by time scale differences among biochemical reactions. Moreover, effects of time delay are also examined. We show that time delay generally enlarges the stability region of oscillations, thereby making the oscillations more sustainable despite parameter changes or noise. The dynamics of the proposed models is robust in terms of stability and period length to the parameter perturbations or

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environment variations. Although we mainly analyze some specific models, the mechanisms identified in this paper are likely to apply to a variety of genetic regulatory systems.

Different models for gene–protein networks have been so far proposed by using circuit and system theoretic models, such as electrical circuits, Boolean networks, Fourier coefficients, Bayesian Networks, differential equations, Petri nets, and Weight matrices [18]. In particular, since gene–protein networks are generally high-dimensional nonlinear systems, nonlinear systems theory seems to be useful for studies on the network dynamics just like such theory has been greatly contributing to understanding the brain dynamics. It can also be expected from the viewpoint of engineering applications of circuits and systems that theoretical studies on gene–protein networks lead to new technological developments such as establishment of biotechnological design principles for synthetic genetic regulatory networks [14], [19] and real implementation of biotechnological devices for sensing and computing.

In Section II, we first describe a dynamical model of genetic regulation for a two-gene system with an autoregulatory feedback loop to design and analyze periodic oscillations, such as circadian rhythms, and the jumping mechanism based on the singular perturbation theory, and we then provide several sufficient conditions to ensure sustained oscillations.

In Section III, we introduce time delay into the model to investigate its effects on the stability region and the period length of the oscillations. We show that this simple model can actually act as a simple genetic oscillator in gene–protein networks. Two examples for an abstract two-gene system and a biological plausible three-gene system are given to numerically demonstrate our theoretical results in Section IV. Finally, we conclude with several summary remarks in Section V.

II. PERIODIC RELAXATION OSCILLATIONS IN A TWO-GENE MODEL

A. A Model With Multiple Time Scales

Mathematical models that extract functional information from observations or experiments are useful for discovering higher order structures of an organizm and gaining deep insights into both static and dynamical behaviors of biological systems [2], [14], [18], [20]–[23].

In this section, we use a simplified abstract two-gene model to qualitatively analyze periodic oscillations, such as circadian rhythms appearing in most organisms with day–night cycles [3], [7], [24], [25]. Fig. 1 shows schematically system structure of our model. Gene Q produces protein $q(t)$, which enhances transcription of itself but represses that of Gene P, whereas the product of Gene P is the protein $p(t)$ that is an activator of Gene Q. To keep the number of variables to minimum, we adopt the following two-variable dynamical system only for $q(t)$ and $p(t)$ concentrations without explicitly expressing mRNA or other related chemicals

$$\dot{p}(t) = -k_p p(t) + \frac{k_1}{q(t) + k_2} \qquad (1)$$

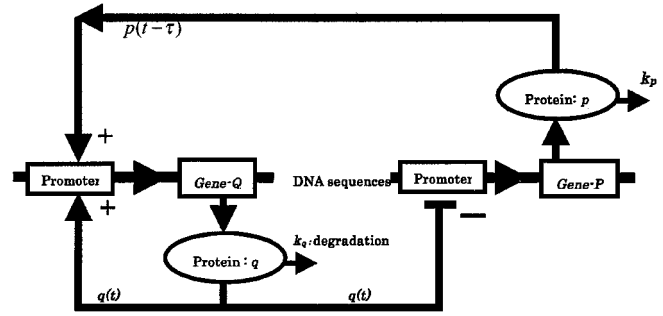$$\epsilon \dot{q}(t) = -k_q q(t) + \frac{q^2(t)}{q^2(t) + k_4} p(t) + k_3 \qquad (2)$$

Fig. 1. A two-gene model of genetic regulatory system with an autoregulatory feedback loop.

where (1) represents total transcription and translation processes of Gene P expression, which are inhibited by protein $q(t)$. Equation (2) represents the dynamics of Gene Q expression, which is enhanced by both $p(t)$ and $q(t)$, where protein $q(t)$ is assumed to form a dimer to activate Gene Q [7]. Equations (1) and (2) can be derived by a statistical thermodynamic–kinetic principle [6], [7], [9]. $p(t)$ and $q(t)$ may be phosphorylated or multimerized when binding to DNA sequences. k_p and k_q/ϵ are the degradation rates (or kinetic rates of decay) for the target proteins, whereas k_1 is the transcription and translation rate for gene P. k_2 is the Michaelis–Menten constant. k_3 and k_4 are lumped parameters that describe the effects of binding or multimerization of proteins, phosphorylation, and other similar phenomena. ϵ is a small positive real number expressing difference of time scales. $\dot{q}(t) = dq(t)/dt$, and $\dot{p}(t) = dp(t)/dt$. The second term of (1) can also be $k_1/[q^2(t) + k_4]$. In this section, we ignore effects of time delay.

The second term of the right-hand side of (2) is the Hill function, representing the nonlinear property with the Hill coefficient equal to 2. This term implies that there is a switch-like phenomenon: the synthesizing rate is drastically increased if q is more than a certain value. In other words, Gene Q has a positive-feedback loop in which the transcription factor q activates its own transcription. Equation (2) for q can also be viewed as an autocatalytic process, which is stimulated or induced by p. Such an autocatalytic process is generally not a single biochemical reaction; rather, it is a series of combined reactions [26]. The parameter ϵ in (2), if sufficiently small, allows us to apply the singular perturbation theory to the model by exploiting the time scale difference between the biochemical reaction processes. In what follows, we assume that the time scale of (2) is much smaller than that of (1). Thus, (1) describes the slow system, whereas (2) represents the fast system.

B. Mechanism of the Relaxation Oscillator

The time-scale difference between (1) and (2) can be exploited by the standard singular perturbation theory. Due to this property of multiple time scales, (1) and (2) can produce a relaxation oscillation.

When $\epsilon \to 0$, the limit system described by (1) and (2) with the time scale t can be expressed as a slow subsystem

$$0 = -k_q q(t) + \frac{q^2(t)}{q^2(t) + k_4} p(t) + k_3 \qquad (3)$$

$$\dot{p}(t) = -k_p p(t) + \frac{k_1}{q(t) + k_2}. \qquad (4)$$

Limit cycle: A->B->D->C->A; Jumping points: B and C
(E: equilibrium point of overall system)
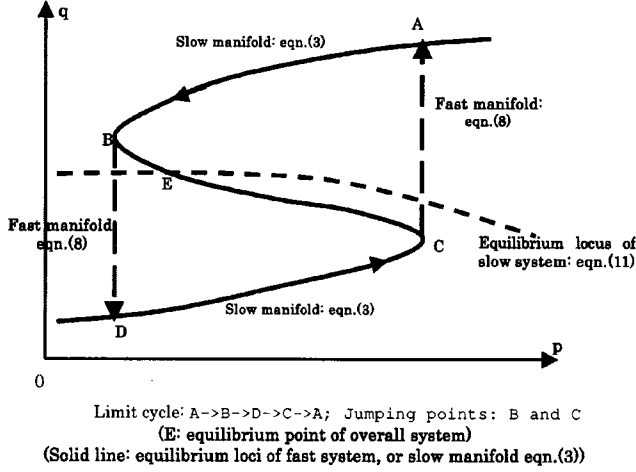(Solid line: equilibrium loci of fast system, or slow manifold eqn.(3))

Fig. 2. A limit cycle generated by a two-gene model.

However, if we rescale (1) and (2) by letting $t = \epsilon\tau$, then we have (5) and (6) with time scale τ

$$q'(\tau) = -k_q q(\tau) + \frac{q^2(\tau)}{q^2(\tau) + k_4} p(\tau) + k_3 \quad (5)$$

$$p'(\tau) = \epsilon\left(-k_p p(\tau) + \frac{k_1}{q(\tau) + k_2}\right) \quad (6)$$

where $q'(\tau) = dq(\tau)/d\tau$ and $p'(\tau) = dp(\tau)/d\tau$. When $\epsilon \to 0$, we have another limit system with the time scale τ, which we refer to as a fast subsystem

$$q'(\tau) = -k_q q(\tau) + \frac{q^2(\tau)}{q^2(\tau) + k_4} p(\tau) + k_3 \quad (7)$$

$$p'(\tau) = 0. \quad (8)$$

Actually, both (3) and (4) and (7) and (8) correspond to zero-order systems of ϵ when substituting $q = q_0 + \epsilon q_1 + \epsilon^2 q_2 + \cdots$ and $p = p_0 + \epsilon p_1 + \epsilon^2 p_2 + \cdots$ into (1) and (2) and (5) and (6), respectively. Fig. 2 shows the two limit systems represented on into the (p, q) plane. Equation (3) corresponds to the slow manifold, whereas (8) generates the fast manifold (or foliation).

Since the Jacobian of (2) for $q(t)$ at any point is J_q/ϵ where

$$J_q = -k_q + \frac{2k_4 q(t)p(t)}{(q^2(t) + k_4)^2} \quad (9)$$

it is easy to confirm that A–B and D–C are stable equilibrium loci (or manifolds) of (2) for q due to $J_q < 0$, whereas B–E–C are unstable equilibria of q due to $J_q > 0$ when p is taken as a parameter [7], [8]. Since the dynamics of q is much faster than that of p, q rapidly approaches its stable equilibrium and moves approximately along the stable equilibrium locus or the slow manifold [i.e., A–B and D–C of (3)] when analyzing the overall dynamics of (1) and (2).

However, the Jacobian matrix J of (1) and (2) for (p, q) is

$$J = \frac{1}{\epsilon}\begin{bmatrix} -\epsilon k_p & -\dfrac{\epsilon k_1}{(q + k_2)^2} \\[2mm] \dfrac{q^2}{q^2 + k_4} & J_q \end{bmatrix}. \quad (10)$$

Therefore, according to the Jacobian matrix J, the stability of (1) and (2) at an equilibrium depends on the eigenvalue of J_q of (2), provided that ϵ is sufficiently small.

When q is taken as a parameter, the stable equilibrium of (1) for p can be described as

$$k_1 - k_p p(t)(q(t) + k_2) = 0 \quad (11)$$

which is derived from (1) by letting $\dot{p} = 0$.

Obviously, when (11) transversally intersects curve A–B or D–C, there is no oscillation; instead there are stable equilibrium points for (1) and (2), according to the eigenvalues of the Jacobian matrix of (10). However, if (11) intersects only curve B–E–C, a limit cycle or a relaxation oscillator exists, as shown schematically in Fig. 2.

When ϵ is sufficiently small, the limit cycle can be approximately described as follows. The flow slowly moves along A–B toward B, the dynamics of which is dominated by the slow system of (1) or (4). When the state is close to B, the flow switches from (3) and (4) to (7) and (8) and moves rapidly from B to D, due to the dominant dynamics of the fast system of (2). Then, the flow continues to move slowly from D to C again along the slow manifold of (3) in the state space. Furthermore, the dynamics switch to the fast dynamics at C and eventually return to A. The period of the limit cycle is approximately equal to the moving time for A → B and D → C, which is determined by the slow system of (1) constrained by (3).

If E is situated between B and C, clearly E is an unstable equilibrium point for the overall system of (1) and (2) and induces the periodic oscillation. On the other hand, if E is located between B and A (or beyond A) or between C and D (or beyond D), E is a stable equilibrium point of the overall system and attracts all trajectories of its neighborhood. Moreover, when E passes through B or C, there is a Hopf bifurcation, which generates or eliminates the periodic oscillation. In particular, the bifurcation is actually singular homoclinic due to the periodic orbit passing through E when $\epsilon \to 0$, and E = B or E = C. Since the dynamics changes between fast and slow systems, where the slow system and the fast system can be viewed as a continuous system and a discrete system respectively, (1) and (2) can also be called a hybrid system [27], [28], [29]. In addition, there may be a complicated orbit, a canard solution [31] that exists only in parameter ranges exponentially small in relation to ϵ when E approaches B or C.

E must be kept between B and C for parameter perturbation so that the oscillation remains stable. Otherwise, this relaxation oscillation will disappear, i.e., the system converges to upper or lower stable equilibrium points when E is between B–A and between C-D respectively; this situation can be used to realize bistability switching. The mechanism of the relaxation oscillation can be viewed as the global dynamics between two attractors of (2). For example, after the ongoing flow reaches C along one attractor D–C of (2) with slow change of variable $p(t)$ in Fig. 2, the stable equilibrium point for $q(t)$ disappears due to the saddle-node bifurcation, which triggers the jumping change; that is, the dynamical flow leaves the attractor D–C and rapidly moves to another attractor A–B of (2). Actually, all fold points, B and C in the slow manifold of (3) where the Jacobian of (2) for $q(t)$ is singular, correspond to saddle-node bifurcation points in the slow subsystem.

The dynamics described above implies that the periodic oscillations are generated by the nonlinearly negative feedback

loop, whereas the jumping dynamics is mainly influenced by time scale difference. This simple model can actually be viewed as a simple genetic oscillator or switch [14], [19]. In addition, when the parameters in (1) and (2) are perturbated, there is no significant change in dynamics (e.g., stability) provided that ϵ is sufficiently small, which implies that (1) and (2) acting as a genetic oscillator or switch are robust for parameter variations or environment variations.

Theorem 2.1: Assume that (1) and (2) have only one equilibrium point E = $(\overline{p}, \overline{q})$. When ϵ is sufficiently small:

if $J_q > 0$, (1) and (2) have a periodic solution around E;

if $J_q < 0$, (1) and (2) have a stable equilibrium point at E.

Proof of Theorem 2.1: When ϵ is sufficiently small, it is easy to show that two eigenvalues for the Jacobian matrix of (1) and (2) are in the form

$$\lambda_1 = \frac{1}{\epsilon} J_q + \mathcal{O}(1) \qquad \lambda_2 = -k_p + \frac{c}{J_q} + \mathcal{O}(\epsilon)$$

where $c = k_1 q^2 / [(q^2 + k_4)(q + k_2)^2]$ and $J_q \neq 0$. λ_1 is positive if $J_q > 0$. Therefore, there is no stable equilibrium point according to the assumption of this theorem. Notice that the equilibrium $(\overline{p}, \overline{q})$ is independent of ϵ.

For the limit system of (3), the equilibria satisfy $p(q) = (k_q q - k_3)(q^2 + k_4)/q^2$. It is easy to show that $dp(\overline{q})/dq < 0$ when $J_q > 0$. On the other hand, $dp(q)/dq > 0$ when $q \rightarrow +\infty$ or $q \rightarrow k_3/k_q$. Therefore, on the basis of graphical analysis, $\overline{q} > k_3/k_q$, and $dp(q)/dq$ has two zero points when $q > k_3/k_q$. Hence, (3) or $k_q q^3 - (k_3 + \overline{p})q^2 + k_q k_4 q - k_3 k_4 = 0$ has three different positive real roots for q.

We then show that there exists a trapping region for (1) and (2). When $q(t) < k_3/k_q$, $\dot{q}(t) > 0$ according to (2), which means $q(t) > k_3/k_q$. Furthermore, when $p(t) < 0$, $\dot{p}(t) > 0$ according to (1), which means $p(t) > 0$. In the same way, we can show that $\dot{p}(t) < 0$ and $\dot{q}(t) < 0$ when $p(t)$ and $q(t)$ are sufficiently large according to (1) and (2) respectively, which prove the existence of a trapping region. Since there is only one unstable equilibrium E, as shown in Fig. 2, with the Poincare–Bendixson Theorem [30], it can be proven that a periodic solution exists for (1) and (2).

If $J_q < 0$, all eigenvalues λ_1 and λ_2 of (1) and (2) have negative real parts, which means that there is only one stable equilibrium point. *End-of-Proof*

Theorem 2.1 implies that there is a limit cycle in the neighborhood of the fast and slow manifolds, as indicated in Fig. 2, provided that the equilibrium loci for the slow and fast systems intersect once at E between B and C. If the intersection E is on line B–A or C–D, there is no limit cycle but a stable equilibrium point, which attracts all flow in its neighborhood.

Theorem 2.1 also suggests that periodic jumping dynamics may exist robustly, provided that there are two different time scale dynamics in the gene–protein network. In addition, when the parameters in (1) and (2) are perturbed, there is no significant change in dynamics (e.g., stability) provided that ϵ is sufficiently small, which implies that (1) and (2) acting as a genetic oscillator or switch is robust for parameter variations or environment variations.
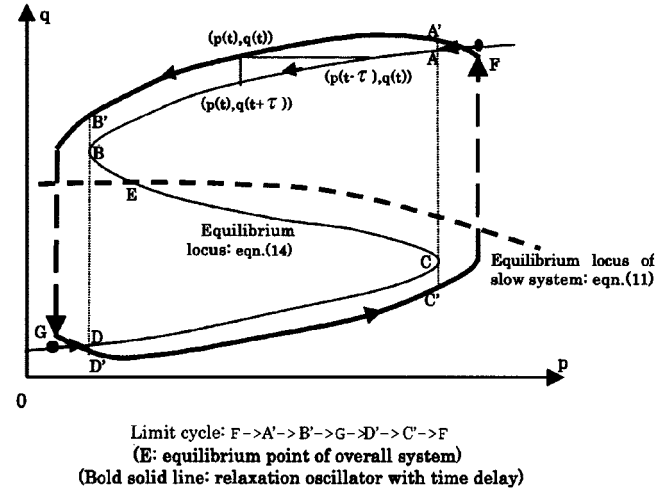

Fig. 3. A limit cycle generated by a two-gene model with time delay.

III. EFFECTS OF TIME DELAY IN THE HYSTERETIC MODEL

One of the key factors affecting the dynamics of gene–protein networks is time delays, which usually exist in transcription, translation, and translocation processes and may significantly influence stability of the overall system, in particular in an eukaryotic cell. In this section, we assume that there is time delay $\tau \geq 0$ only for the slow variable p and that the time delay for the fast variable q is so small that it can be ignored. Then (1) and (2) become

$$\dot{p}(t) = -k_p p(t) + \frac{k_1}{q(t) + k_2} \tag{12}$$

$$\epsilon \dot{q}(t) = -k_q q(t) + \frac{q^2(t)}{q^2(t) + k_4} p(t - \tau) + k_3. \tag{13}$$

By the same analysis as (1) and (2), we have the following slow manifold for variables $(p(t - \tau), q(t))$ of (12) and (13)

$$0 = -k_q q(t) + \frac{q^2(t)}{q^2(t) + k_4} p(t - \tau) + k_3. \tag{14}$$

However, the equilibrium locus of the slow system for (12) is the same as that for (11).

Fig. 3 is a schematic illustration of the oscillation of the model for small τ, where the outer loop is the periodic orbit for $(p(t), q(t))$ and the inner curve is (14) for $(p(t - \tau), q(t))$ that is the same as Fig. 1 of $(p(t), q(t))$ without time delay. Since ϵ is sufficiently small, $(p(t - \tau), q(t))$ from all initial values are strongly attracted to the slow manifold of (14) if the equilibrium of the overall system is unstable. As demonstrated in Fig. 3, any point $(p(t), q(t))$ for the system can be drawn by two points $(p(t - \tau), q(t))$ and $(p(t), q(t + \tau))$ on the curve of (14). Moreover, (12) and (13) can be approximated by ordinary differential equations (ODE) by replacing $p(t - \tau) \approx p(t) - \tau \dot{p}(t)$ when τ is sufficiently small.

The orbit with the time delay shown in Fig. 3 generally has a longer period, which is due to a longer movement on the slow manifold, than the orbit without time delay shown in Fig. 1. Furthermore, the stability region of this longer relaxation oscillation is also enlarged because of the time delay. Specifically, the oscillation can be sustained even if E moves on B–A or C–D, in

contrast to the stability region between B and C without the time delay in Fig. 2. Therefore, the time delay generally increases the robustness of sustained oscillations, which implies that time delay may suppress an effect of noise in biological systems. On the other hand, the time delay can be used to adjust the period length for a synthetic oscillator because of the dependence of the period length on the time delay. Next, we give the conditions of oscillations with time delay.

The characteristic equation of (12) and (13) at the unique equilibrium point $E = (\overline{p}, \overline{q})$ can be obtained by first linearizing (12) and (13) around E and then inserting $p(t) = c_p e^{\lambda t}$, $q(t) = c_q e^{\lambda t}$ into the linear equations [32]

$$\lambda^2 + \frac{a}{\epsilon} \lambda + \frac{b}{\epsilon} + \frac{c}{\epsilon} e^{-\lambda\tau} = 0 \qquad (15)$$

where $a = \epsilon k_p - J_q$, $b = -J_q k_p$, and $c = k_1 q^2/[(q^2 + k_4)(q + k_2)^2]$ at $E = (\overline{p}, \overline{q})$, and $\lambda \in \mathcal{C}$. The roots of the transcendental equation for λ determines the stability of the equilibrium point E. If real parts of all roots are negative, E is an stable equilibrium and there is no oscillation. On the other hand, if there exists a root with a positive real part, the oscillation exists. In other words, the bifurcations occur when $\lambda = jv$ is a root of (15)

$$b - \epsilon v^2 + c \cos(v\tau) = 0 \qquad (16)$$

$$av - c \sin(v\tau) = 0. \qquad (17)$$

Clearly, when $\tau = 0$, from (16) and (17) or (15) we can derive $a = 0$ and $v^2 = (b + c)/\epsilon$, or $J_q = \epsilon k_p$ and $v = \pm\sqrt{c/\epsilon - k_p^2}$. In other words, without the time delay, the bifurcation condition is $\lim_{\epsilon \to 0} J_q = 0$, which is consistent with Theorem 2.1.

For $\tau > 0$ and $a \neq 0$, by eliminating terms of sin and cos, we have

$$v^4 + \frac{\overline{a}}{\epsilon^2} v^2 + \frac{\overline{b}}{\epsilon^2} = 0 \qquad (18)$$

where $\overline{a} = a^2 - 2b\epsilon$ and $\overline{b} = b^2 - c^2$. When ϵ is sufficiently small and $|c| > |b|$, the real solutions of (18) can be written as

$$v = \pm \left[-\frac{\overline{a}}{2\epsilon^2} + \left(\frac{\overline{a}^2}{4\epsilon^4} - \frac{\overline{b}}{\epsilon^2} \right)^{1/2} \right]^{1/2}$$

$$= \pm \left[-\frac{\overline{a}}{2\epsilon^2} + \left(\frac{\overline{a}}{2\epsilon^2} - \frac{\overline{b}}{\overline{a}} + \mathcal{O}(\epsilon) \right) \right]^{1/2}.$$

Therefore, the critical values for $\overline{v}$ and $\overline{\tau}_k$ are

$$\overline{v} = \pm \left[\frac{c^2 - b^2}{a^2} + \mathcal{O}(\epsilon) \right]^{1/2} = \pm\sqrt{c^2 - b^2}/a + \mathcal{O}(\epsilon)$$

and for $k = 0, 1, 2, \ldots$

$$\overline{\tau}_k = \frac{1}{v} \left[2k\pi + \arccos\left(\frac{\epsilon v^2 - b}{c} \right) \right]$$

$$= \frac{|a|}{\sqrt{c^2 - b^2}} \left[2k\pi + \arccos\left(-\frac{b}{c} \right) \right] + \mathcal{O}(\epsilon) \qquad (19)$$

where the range of arccos is $[0, \pi]$. When $|c| < |b|$, clearly there is no real solution of (18), which means that no Hopf bifurcation could occur for any τ.

On the other hand, from (15), we have

$$\left(\frac{d\lambda}{d\tau} \right)^{-1} = \frac{2\epsilon\lambda + a}{c\lambda} e^{\lambda\tau} - \frac{\tau}{\lambda}. \qquad (20)$$

Since $\partial(Re\lambda)/\partial\tau = |\lambda|^2 Re[(d\lambda/d\tau)^{-1}]$, by substituting (16) and (17) into the real part of (20) at $\lambda = jv$, we obtain

$$\left. \frac{\partial(Re\lambda)}{\partial\tau} \right|_{\lambda=jv} = v^2 \frac{2\epsilon^2 v^2 - 2\epsilon b + a^2}{c^2} = \frac{v^2 a^2}{c^2} + \mathcal{O}(\epsilon).$$

Thus, when ϵ is sufficiently small, $\partial(Re\lambda)/\partial\tau|_{\lambda=jv} > 0$, which implies that real part of any λ moves to the right half plane (or positive region) for increasing τ when λ is on the imaginary axis. In other words, time delay destabilizes the equilibrium E. The unstable equilibrium remains unstable but the stable equilibrium may become unstable with the increase of time delay. On the other hand, as the same way as the argument of (1) and (2), it is easy to verify that (12) and (13) have a trapping region around E. Therefore, we have the following theorem by summarizing the discussions in this section.

Theorem 3.1: Assume that (12) and (13) have only one equilibrium point $E = (\overline{p}, \overline{q})$. When ϵ is sufficiently small:

if $J_q > 0$, (12) and (13) have an oscillation solution around E for any nonnegative τ;

if $J_q < 0$ and $|c| \geq |b|$, (12) and (13) have an oscillation solution around E when $\tau > \overline{\tau}_0$;

if $J_q < 0$ and $|c| < |b|$, (12) and (13) have a stable equilibrium point at E for any nonnegative τ.

$\overline{\tau}_0$ in Theorem 3.1 is the first bifurcation value in (19) at $k = 0$. Theorem 3.1 indicates that time delay enlarges the stability region of oscillation, which includes $J_q < 0$. Although Theorems 2.1–3.1 are proven for the specific systems (1) and (2) and (12) and (13), they can be applied to other relaxation oscillation systems with little modification.

IV. NUMERICAL EXAMPLES FOR TWO-GENE AND THREE-GENE SYSTEMS

In this section, we first use a numerical example for the abstract system, (12) and (13) with and without time delay to verify the theoretical results.

Example 4.1:

$$\dot{p}(t) = -k_p p(t) + \frac{k_1}{q(t) + k_2} \qquad (21)$$

$$\epsilon\dot{q}(t) = -k_q q(t) + \frac{q^2(t)}{q^2(t) + k_4} p(t - \tau) + k_3 \qquad (22)$$

where $k_p = 1$, $k_q = 1$, $k_1 = 15$, $k_2 = 0.2$, $k_3 = 0.1$, and $k_4 = 10$. All variables are positive numbers.

For the Case of $\tau = 0$: There is only one equilibrium $(\overline{p}, \overline{q}) = (7.6831, 1.4787)$ of the overall system, and it is unstable, independent of ϵ, and $J_q = 0.53 > 0$. Fig. 4 is the phase portrait for $\epsilon = 0.01$ based on numerical simulation. Fig. 5 indicates the time courses for $q(t)$ and $p(t)$, which demonstrate the relaxation oscillation. The period T is approximately 3, which is scaled by a proper constant, e.g., the constant should be $24/3 \approx 8$ h for explaining a circadian rhythm. As shown in Fig. 5, the dynamics of $q(t)$ are not only periodic but also change drastically at some points, as in many biological phenomena of living organisms [2], [15]. There also is a phase difference between the proteins $q(t)$ and $p(t)$, and the maximum of $q(t)$ follows the peak of $p(t)$ by a short time, as indicated in Fig. 5.

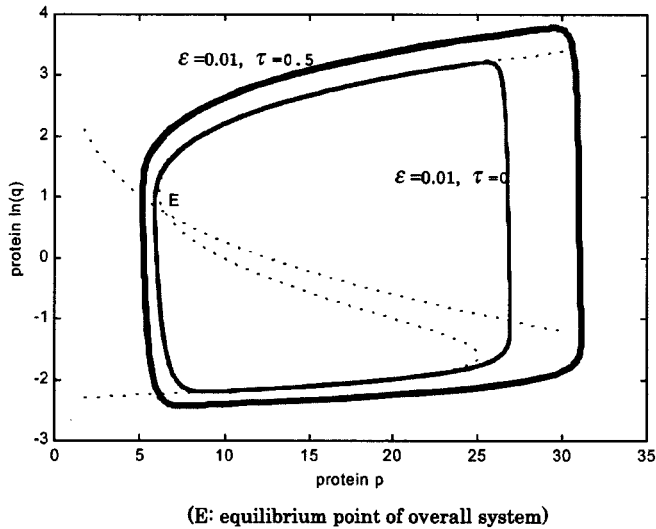(E: equilibrium point of overall system)

Fig. 4.   A limit cycle obtained by numerical simulation for $\epsilon = 0.01$ with $\tau = 0$ and $\tau = 0.5$.
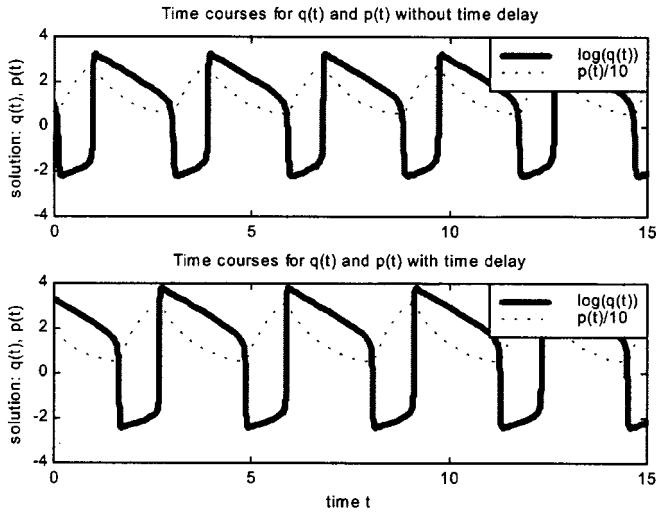

Fig. 5.   Time evolutions of $q(t)$ and $p(t)$ for $\epsilon = 0.01$ with $\tau = 0$ and $\tau = 0.5$.

Due to the absence of experiment data, all parameters for concentrations and kinetic constants in this example are appropriately chosen to satisfy the conditions of Fig. 2 and yield a periodic oscillation.

Fig. 6 shows limit cycles when ϵ is taken as a varying parameter. Evidently, when the value of ϵ increases, there are no clear jumping changes, and the dynamics are not always attracted on the slow manifold. Conversely, if ϵ decreases, the flow is almost restricted to the slow manifold with the fast manifold acting as two bridges that connect the trajectory between the upper and lower parts of the slow manifold [or two attractors of the fast system (22) for $q(t)$]. Fig. 6 indicates that the jumping dynamics are mainly contributed by the time scale difference ϵ between the fast and slow systems.

For the Case of $\tau = 0.5$:   When the time delay is considered, $(\overline{p}, \overline{q}) = (7.6831, 1.4787)$ is also the equilibrium point for the overall system, which is unstable for any time delay τ according to Theorem 3.1 (due to $J_q = 0.53 > 0$). Fig. 4 shows the phase portrait for $\epsilon = 0.01$ with $\tau = 0.5$ by numerical simulation.
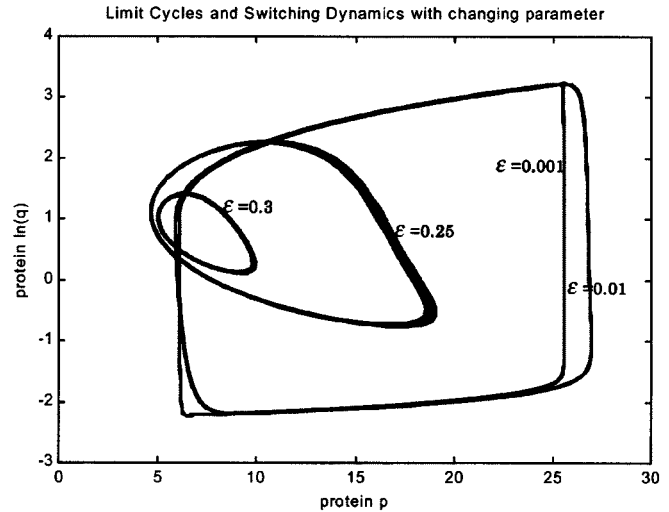

Fig. 6.   Limit cycles and jumping dynamics for $\epsilon = 0.001, 0.01, 0.25, 0.3$ with $\tau = 0$.
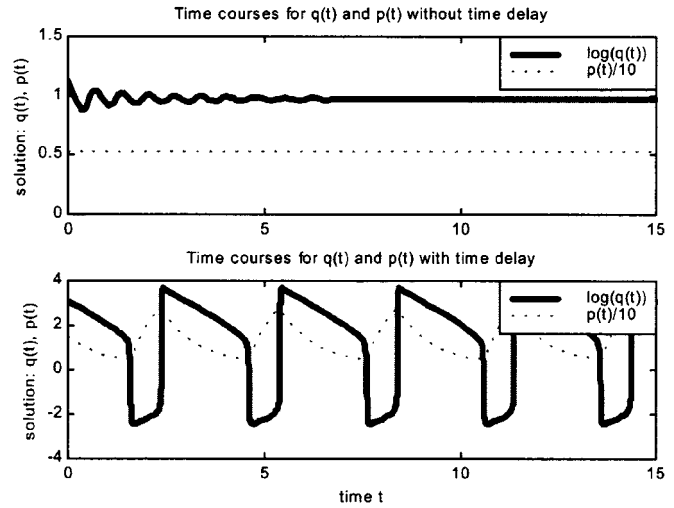

Fig. 7.   Time evolutions of $q(t)$ and $p(t)$ for $\epsilon = 0.01$ and $k_4 = 7.5$ with $\tau = 0$ and $\tau = 0.5$.

Fig. 5 indicates the time courses for $q(t)$ and $p(t)$. Clearly, not only the amplitude but also the period T are larger than those of the case without the time delay.

Moreover, the stability of the oscillator is also enhanced due to the time delay. Even if the overall equilibrium is on the line B–A or C–D, of which the system without the time delay stays at an equilibrium point, there may exist a sustained oscillation. For example, when $k_4 = 7.5$, the equilibrium is E $= (5.0501, 4.1965)$, where $J_q = -0.4958 < 0$. According to Theorem 3.1, there is no oscillation for the system with $\tau = 0$ where $J_q < 0$, but the system with $\tau = 0.5$ still has the relaxation oscillation, as shown in Fig. 7. Actually, the numerical simulation shows that the stability ranges of the oscillation for parameter k_4 are:

- $7.6 \le k_4 \le 14.8$ if $\tau = 0$
- $4.2 \le k_4 \le 15.8$ if $\tau = 0.5$

which confirms that the time delay enlarges the stability region of the oscillation.

Next, we examine a biological plausible three-gene model shown in Fig.8 where proteins p_1 and p_3 form a heterodimer
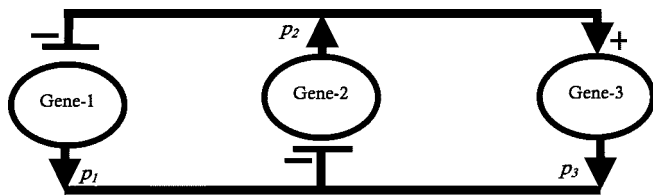
Fig. 8. A three-gene model of genetic regulation. Proteins p_1 and p_2 form a heterodimer to inhibit gene-2, whereas protein p_3 forms a homodimer to activate gene-3 and inhibit gene-1.

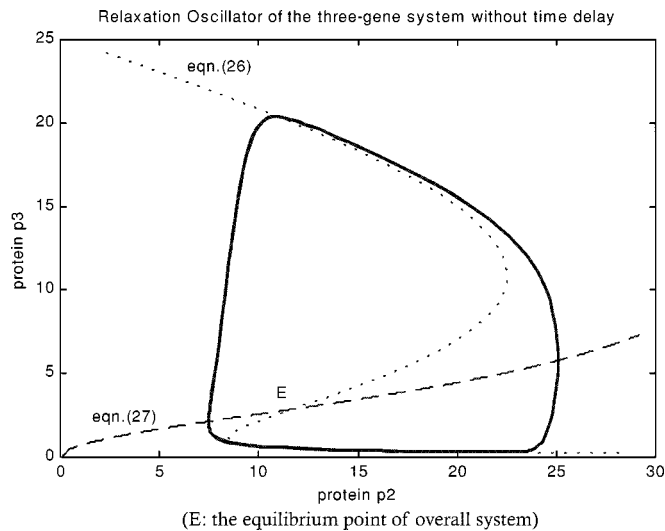(E: the equilibrium point of overall system)

Fig.9. A limit cycle of the three-gene model obtained by numerical simulation with $\epsilon = 0.01$ and $\tau = 0$.

to inhibit gene-2, whereas protein p_2 forms a homodimer to activate gene-3 and inhibit gene-1.

Example 4.2: Assume that the productions of proteins p_1 and p_2 are much faster than that of protein p_3

$$\epsilon \dot{p}_1(t) = \frac{k_1}{1 + a_1 p_2^2(t)} - d_1 p_1(t) + b_1 \qquad (23)$$

$$\epsilon \dot{p}_2(t) = \frac{k_2}{1 + a_2 p_1(t) p_3(t - \tau)} - d_2 p_2(t) + b_2 \qquad (24)$$

$$\dot{p}_3(t) = \frac{k_3 p_2^2(t)}{1 + a_3 p_2^2(t)} - d_3 p_3(t) + b_3 \qquad (25)$$

where $\epsilon = 0.01$, $d_1 = d_2 = d_3 = 0.04$, $b_1 = b_2 = b_3 = 0.004$, $k_1 = 4, k_2 = 1, k_3 = 0.08$, and $a_1 = 1, a_2 = 1/16, a_3 = 0.05$. All variables are positive. τ is the time delay.

Assume $\tau = 0$. From (23) and (24) we have the equilibrium loci of the fast system

$$p_3 = \frac{d_1(1 + a_1 p_2^2)(k_2 + b_2 - d_2 p_2)}{a_2(d_2 p_2 - b_2)(k_1 + b_1(1 + a_1 p_2^2))}. \qquad (26)$$

On the other hand, the equilibrium loci of the slow system can be derived from (25)

$$p_3 = \frac{k_3 p_2^2}{d_3(1 + a_3 p_2^2)} + \frac{b_3}{d_3}. \qquad (27)$$

Fig.9 shows the limit cycle as well as the equilibrium loci for both the fast system and the slow system by numerical simulation. Clearly, the relaxation oscillation is mainly due to the time scale difference and the hysteresis of the slow manifold. In the same way as the simulation of (21) and (22), we can obtain the

time evolutions and the effects of the time delay, which are similar to Example 4.1.

In this paper, although we focus on investigating nonlinear dynamics of two-gene system (12) and (13) and three-gene system (23)–(25), all of the theoretical results can also be applied to other models with hysteretic properties.

V. CONCLUSION

We used a two-variable dynamical model of genetic regulation to analyze the basic mechanism of the periodic oscillation by the singular perturbation theory.

A significant property in gene expression systems is the existence of many different time scales for the gene regulatory dynamics. In this paper, we exploited this property of fast and slow dynamics and proved that there exists a relaxation oscillator with jumping dynamics provided that the difference in time scales is sufficiently large. For individual biochemical gene–protein reactions, the slow dynamics with large time scales is mainly constructed by the transcription and translation processes, whereas the fast dynamics with small time scales may be biochemical reactions like phosphorylation, dimerization, and binding. We showed that the periodic oscillations are generally produced by the nonlinear feedback loops in the gene regulatory systems, whereas the jumping dynamics are mainly caused by time scale differences among the biochemical reactions. Since the time delay may significantly influence gene expression and periodic oscillations [8], we also analyzed the effects of the time delay on the stability of the relaxation oscillator in the genetic regulatory systems, and showed that the time delay generally not only increases the period but also enlarges the stability region of the oscillation, thereby making the oscillation robust for parameter variations or noise. Further, the time delay can be used to control the period of the oscillation, as indicated in Fig. 4.

The models of the relaxation oscillation display robustness in dynamics to parameter perturbations or environment variations due to extremely stable limit cycles. Although this paper mainly analyzes the specific systems (1) and (2) and (12) and (13), the results and the mechanisms identified in this paper can be applied to a variety of genetic regulatory systems. To demonstrate our theoretical results, we used two examples that include the time scale difference as a varying parameter for the numerical simulation with the time delay. However, our model does not include stochastic thermal fluctuations in molecule numbers although such fluctuations may be important for the dynamics of gene expression [7]. It is a future problem to compare the oscillation properties in our model with those in models of neural systems and other biological systems to derive novel insights or properties of genetic networks. In addition, since our models are relatively abstract and are not derived from particular biological examples, it is also important to investigate if or not a real gene network really adopts such fast–slow dynamics for emergence of oscillations.

REFERENCES

[1] B. C. Goodwin, "Oscillatory behavior in enzymatic control processes," *Adv. Enzyme Regul.*, vol. 3, pp. 425–438, 1965.

[2] L. Glass and S. A. Kauffman, "The logical analysis of continuous, nonlinear biochemical control networks," *J. Theor. Biol.*, vol. 39, no. 1, pp. 103–129, 1973.

[3] J. C. Dunlap, "Molecular bases for circadian clocks," *Cell*, vol. 96, no. 2, pp. 271–290, 1999.

[4] A. Goldbeter, "A model for circadian oscillations in the *Drosophila* period protein (PER)," *Proc. R. Soc. Lond.*, vol. B.261, no. 1362, pp. 319–324, 1995.

[5] J. Leloup and A. Goldbeter, "Modeling the molecular regulatory mechanism of circadian rhythms in *Drosophila*," *BioEssays*, vol. 22, no. 1, pp. 84–93, 2000.

[6] A. Keller, "Model genetic circuits encoding autoregulatory transcription factors," *J. Theor. Biol.*, vol. 172, pp. 169–185, 1995.

[7] P. Smolen, D. A. Baxter, and J. H. Byrne, "Frequency selectivity, multistability, and oscillations emerge from models of genetic regulatory systems," *Amer. J. Physiol.*, vol. 274, no. 2, pp. c532–c542, 1998.

[8] ——, "Mathematical modeling of gene networks review," *Neuron*, vol. 26, no. 3, pp. 567–580, 2000.

[9] D. M. Wolf and F. H. Eeckman, "On the relationship between genomic regulatory element organization and gene regulatory dynamics," *J. Theor. Biol.*, vol. 195, no. 2, pp. 167–186, 1998.

[10] P. Ruoff, M. Vinsjevik, S. Mohsenzadeh, and L. Rensing, "The Goodwin model: Simulating the effect of cycloheximide and heat shock on the sporulation rhythm of *Neurospora crassa*," *J. Theor. Biol.*, vol. 196, no. 4, pp. 483–494, 1999.

[11] N. Barbai and S. Leibler, "Biological rhythms: Circadian clocks limited by noise," *Nature*, vol. 403, no. 6767, p. 267, 2000.

[12] J. Hasty, J. Pradines, M. Dolnik, and J. J. Colins, "Noise-based switches and amplifiers for gene expression," *Proc. Natl. Acad., Sci. USA*, vol. 97, no. 5, pp. 2075–2080, 2000.

[13] J. Hasty, F. Isaacs, M. Dolnik, D. McMillen, and J. J. Colins, "Designer gene networks: Toward fundamental cellular control," *Chaos*, vol. 11, no. 1, pp. 207–220, 2001.

[14] T. S. Gardner, C. R. Cantor, and J. J. Collins, "Construction of a genetic toggle switch in *Escherichia coli*," *Nature*, vol. 403, no. 20, pp. 339–342, 2000.

[15] T. Mestl, E. Plahte, and S. W. Omholt, "A mathematical framework for describing and analyzing gene regulatory networks," *J. Theor. Biol.*, vol. 176, no. 2, pp. 291–300, 1995.

[16] J. Cronin, *Mathematical Aspects of Hodgkin–Huxley Neural Theory*. Cambridge, MA: Cambridge Univ. Press, 1987.

[17] E. M. Izhikevich, "Neural excitability, spiking and bursting," *Int. J. Bifurcation Chaos*, vol. 10, no. 6, pp. 1171–1266, 2000.

[18] H. McAdams and L. Shapiro, "Circuit simulation of genetic networks," *Science*, vol. 269, pp. 650–656, 1995.

[19] M. B. Elowitz and S. Leibler, "A synthetic oscillatory network of transcriptional regulators," *Nature*, vol. 403, no. 20, pp. 335–338, 2000.

[20] T. Chen, H. L. He, and G. M. Church, "Modeling gene expression with differential equations," in *Proc. 1999 Pacific Symp. Biocomputing*, Honolulu, HI, 1999, pp. 29–40.

[21] R. Somogyi and C. Sniegoski, "Modeling the complexity of genetic networks: Understanding multigenic and pleiotropic regulation," *Complexity*, vol. 1, pp. 45–63, 1996.

[22] D. Weaver, C. Workman, and G. Stormo, "Modeling regulatory networks with weight matrices," in *Proc. 1999 Pacific Symp. Biocomputing*, Honolulu, HI, 1999, pp. 113–123.

[23] G. Yagil and E. Yagil, "On the relation between effector concentration and the rate of induced enzyme synthesis," *Biophys. J.*, vol. 11, pp. 11–27, 1971.

[24] K. Stokkan, S. Yamazaki, H. Tei, Y. Sakaki, and M. Menaker, "Entrainment of the circadian clock in the liver by feeding," *Science*, vol. 291, no. 5530, pp. 490–493, 2001.

[25] S. Yamazaki, R. Numano, M. Abe, A. Hida, R. Takahashi, M. Ueda, G. D. Block, Y. Sakaki, M. Menaker, and H. Tei, "Resetting central and peripheral circadian oscillators in transgenic rats," *Science*, vol. 288, no. 5466, pp. 682–685, 2000.

[26] G. Houart, G. Dupont, and A. Goldbeter, "Bursting, chaos and birhythmicity originating from self-modulation of the inositol 1, 4, 5-trisphosphate signal in a model for intracellular Ca^{2+} oscillations," *Bull. Math. Biol.*, vol. 61, no. 3, pp. 507–530, 1999.

[27] J. Guckenheimer and S. Johnson, *Planar Hybrid Systems. Hybrid Systems*, P. Antsaklis *et al.*, Eds. New York: Springer-Verlag, 1995.

[28] J. Kevorkian and J. D. Cole, *Multiple Scale and Singular Perturbation Methods*. New York: Springer-Verlag, 1996.

[29] L. Chen and K. Aihara, "Stability and bifurcation analysis of differential–difference–algebraic equations," *IEEE Trans. Circuits Syst. I*, vol. 48, pp. 308–326, Mar. 2001.

[30] J. Guckenheimer and P. Holmes, *Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields*. New York: Springer-Verlag, 1983.

[31] J. Guckenheimer, K. Hoffman, and W. Weckesser, "Numerical computation of canards," *Int. J. Bifurcation Chaos*, vol. 10, no. 12, pp. 2669–2688, 2000.

[32] N. Buric and D. Todorovic, "Dynamics of delay-differential equations modeling immunology of tumor growth," *Chaos Solitons Fractals*, vol. 13, no. 4, pp. 645–655, 2002.

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