Dynamics of gene regulatory networks with cell division cycle

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This paper focuses on modeling and analyzing the nonlinear dynamics of gene regulatory networks with the consideration of a cell division cycle with duplication process of DNA, in particular for switches and oscillators of synthetic networks. We derive two models that may correspond to the eukaryotic and prokaryotic cells, respectively. A biologically plausible three-gene model (*lac*, *tetR*, and *cI*) and a repressilator as switch and oscillator examples are used to illustrate our theoretical results. We show that the cell cycle may play a significant role in gene regulation due to the nonlinear dynamics of a gene regulatory network although gene expressions are usually tightly controlled by transcriptional factors.

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I. INTRODUCTION

Recent advances in genomic science have made the quantitative analysis of DNA-protein interactions realistic due to the progress of experiments and measurements, in contrast to conventional qualitative study. Nonlinear phenomena in cellular dynamics, such as biochemical oscillations and gene expression multistability have extensively been investigated by many mathematical models [1–4]. In particular, for synthetic networks, several simple genetic networks have been successfully constructed experimentally according to theoretical models, i.e., a genetic toggle switch [5], a repressilator [6], and other biocircuits [7,8]. Data in these experiments are consistent with the predictions of mathematical models; this implies that theoretical models are powerful tools for designing and analyzing gene regulatory networks. Such simple models represent a first step towards engineering cellular control by manipulating and monitoring biological processes at the DNA level, and not only can be used as building blocks to synthesize the artificial biological systems, but also have great potential for biotechnological and therapeutic applications [8–19].

On the other hand, since the proteins and genes are dynamically interconnected with each other in a cell, a cell division cycle is naturally thought to have a significant influence on the cellular dynamics, which is neither clearly understood nor well investigated despite growing knowledge about the cell division mechanism and certain qualitative features of molecular fluctuations [8,20–22].

A typical cycle for both eukaryotic and prokaryotic cells is growth and division. Growth implies the buildup of new molecules in a cell and the associated increase in its mass and volume, while division means pinching off of two almost equally sized daughter cells, which is usually a much faster process in contrast to the growth [23,24]. The genome together with associated proteins must be doubled in size with extraordinary precision in preparation for cell division. A cycle of most eukaryotes is composed of four stages: G1 (gap) phase in which size of the cell is increased by constantly producing RNA and synthesizing protein, S phase in which DNA synthesis and duplication occur, G2 (gap) phase in which the cell continues to produce new proteins and grows in size, and M (mitosis) phase in which chromosomes segregate and cell division takes place. In particular, the genome is constantly kept in the G1, G2, and M phases, but duplicated in the S phase which lasts shorter than the cell volume growth process and much longer than the cell division instant. The time period of a cell cycle in most mammalian cells is on the order of 12–24 h, whereas bacteria by contrast may divide every 20–30 min and yeast cells or other protozoans may take 6–8 h [25]. Since the cell volume and the DNA number must grow by a factor of 2 between successive divisions in order to ensure that the mass of the two daughter cells will nearly equal that of the mother cell, the concentrations or the numbers of molecules in the cell inevitably depend on dynamics of the cell cycle, which in turn have a significant effect on the dynamics of gene-protein networks owing to such dynamical fluctuations of the cell cycle.

Recently, it was also found that there is a functional pathway linking cell division and the circadian clock by the experiment in murine-regenerating liver [26]. In addition to cell cycles, stochastic noise [20,22,27,28] and time delays [12,13] are also important factors, which may affect the performance of the entire cellular system. Analyzing the effects of cell cycles on gene switches and oscillators is important not only for designing synthetic gene networks but also for understanding cellular dynamics of biological systems, because both gene switches and oscillators are thought to be essential minimal modules in living organisms [15].

This paper aims at modeling and analyzing the nonlinear dynamics of gene regulatory networks with the consideration of a cell cycle, in particular for switches and oscillators of synthetic networks. The number of chemicals, e.g., proteins, in a cell is partly controlled by the cell cycle, and dilution

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due to partition into daughter cells plays a significant role in keeping the chemical numbers low [22]. Specifically, although the chemical numbers may increase due to the effect of the cell cycle, a cell division cycle mainly accelerates the degradation of the chemical concentrations in a cell. In particular, when the gene network is near a stability boundary, a cell cycle as a degradation factor may significantly change the dynamics both qualitatively and quantitatively. As shown in this paper, a cell division cycle can be viewed as an external periodic force for the inherent autonomous dynamics of genetic networks. Depending on the frequencies and coupling of external and internal oscillations, there may exist periodic, quasi-periodic, or even resonant dynamics that are generated by synchronization of the two oscillators. With growth of a cell, the DNA or gene numbers can be approximately assumed to change rapidly or smoothly, depending on cell types and the initial DNA or gene numbers. In this paper, we derive two models that may correspond to the eukaryotic and prokaryotic cells, respectively. We show that the cell cycle may play a significant role in gene regulation due to the nonlinear relation among the cell volume, the DNA number, and gene regulatory network although gene expressions are usually tightly controlled by transcriptional factors.

This paper is organized as follows. We first give a general description of gene regulatory networks with emphasis on the transcription and translation processes by using nonautonomous differential equations, and transform it into two specific models for cases with smooth and rapid changes of the gene numbers by autonomous ordinary differential equations (ODEs) and impulsive differential equations (IDEs), respectively. Then, we briefly examine the basic properties of periodic solutions and their stability by constructing a Poincaré map. As an implementation example, we design a biologically plausible three-gene synthetic model with *lac*, *tetR*, and *cI*, and derive its differential equations. The first numerical experiment is the analysis of a genetic switch with *lac* and *tetR* and a genetic oscillator with *lac*, *tetR*, and *cI* when the gene numbers are approximately assumed to smoothly change with the cell growth, as in a prokaryotic cell. In contrast, the second numerical experiment is the case for the rapid change of the gene numbers, as in a eukaryotic cell. Besides, we also examine the effects of a cell cycle on the Repressilator [6,12] by numerical simulation. Finally, we give several general remarks to conclude this paper.

II. GENE REGULATORY NETWORKS WITH CELL DIVISION CYCLE

A. Network model

The gene regulatory systems of living organisms are so complicated that any mathematical model has limitations to certain functions. Before defining gene regulatory networks, we make several assumptions.

Assumption II.1. Transcription and translation processes evolve on a time scale that is much slower than those of protein binding, dimerization, phosphorylation, or other chemical modification reactions.

In fact, for most eukaryotic and prokaryotic cells, transcription and translation processes are on a time scale of minutes, whereas the binding, dimerization, or other chemical modification reactions are generally less than a second. Therefore for gene regulatory networks, all chemical reactions are usually reduced to transcription and translation processes [9,12,22].

Besides the transcriptional factors, transcription and translation rates are mainly affected by the numbers of RNA polymerases and ribosomes, which are all house-keeping molecules in a cell and are thought to increase approximately with the cell volume.

Let $m(t)=(m_1(t),\ldots,m_n(t))\in\mathcal{R}^n$ and $p(t)=(p_1(t),\ldots,p_n(t))\in\mathcal{R}^n$ be the numbers of mRNAs and the corresponding proteins in a cell, respectively. In this paper, $m(t)$ and $p(t)$ are approximately taken as real numbers. Then, we have the following general equations [12] for gene regulatory networks in a cell by ODEs in terms of mRNAs $m(t)$ and proteins $p(t)$ according to the law of mass action.

$$\dot{m}(t) = Nu(t)f\left(\frac{p(t)}{v(t)}\right) - K_m m(t), \tag{1}$$

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

where $N=\mathrm{diag}(N_1,\ldots,N_n)$ is a positive diagonal matrix that represents the numbers of genes at the beginning of the cell growth phase, while $u(t)\in\mathcal{R}$ is the DNA number factor so that $Nu(t)$ is the numbers of genes at instant t, thereby $1\leqslant u(t)\leqslant 2$. $K_m=\mathrm{diag}(k_{m1},\ldots,k_{mn})\in\mathcal{R}^{n\times n}$ and $K_p=\mathrm{diag}(k_{p1},\ldots,k_{pn})\in\mathcal{R}^{n\times n}$ are positive diagonal matrices that represent the degradation rates for mRNAs and proteins, respectively. $f(p)=(f_1(p),\ldots,f_n(p))\in\mathcal{R}^n$ is a vector of synthesis rates for transcription, and is generally nonlinear. $S_p=\mathrm{diag}(s_1,\ldots,s_n)\in\mathcal{R}^{n\times n}$ is also a positive diagonal matrix, representing translation rates. Define the cell volume factor as $v(t)=V(t)/V_0\in\mathcal{R}$, where $V(t)$ is the host cell volume at instant t and V_0 is the host cell volume at the beginning of its growth phase, thereby $1\leqslant v(t)\leqslant 2$. For the period from the beginning of cell growth to the cell division, Eq. (1) represents the transcription reaction whereas Eq. (2) stands for the translation process. $\dot{m}(t)=dm(t)/dt$ and $\dot{p}(t)=dp(t)/dt$ for all t except division instants, while the volume and the numbers of chemicals all halve, i.e., $v(t)\to v(t)/2$, $u(t)\to u(t)/2$, $m(t)\to m(t)/2$, and $p(t)\to p(t)/2$ at division instant t.

According to Assumption II.1, all biochemical reactions except transcription and translation processes are reduced into Eqs. (1) and (2) due to the adiabatic condition of slow and fast dynamics [12]. f is the synthesis term that is combined with the effects of binding, multimerization, and other chemical modification reactions with conservation conditions, such as the numbers of binding sites on DNA, and depends on the chemical concentrations [8], e.g., $p(t)/v(t)$ according to the law of mass action. Actually, we can easily verify that Eqs. (1) and (2) are consistent with standard forms of rate equations [29] where $Nu(t)/v(t)$ is the concentration of genes. We will use a three-gene model to show this fact in the next section. In this paper, a chemical means a molecule a complex in a cell.

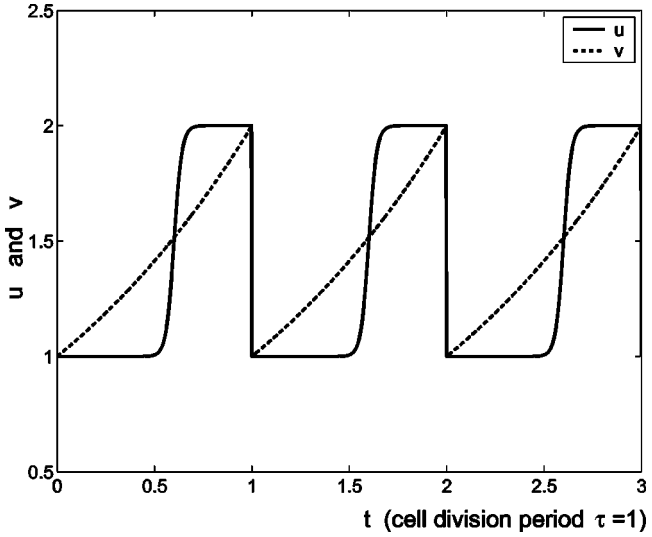

FIG. 1. The cell volume factor $v(t)$ and the DNA number factor $u(t)$ for $t_d=0.6$ and $\gamma=50$.

In a eukaryotic cell, there is usually one copy for each gene at the beginning of a cell growth phase, i.e., $N=\mathrm{diag}(1,1,\ldots,1)$. However, for bacteria, there may exist multiple DNA plasmids per cell, e.g., as many as one hundred plasmids [8,25,30,31], which implies $N_i=1-100$ for $i=1,\ldots,n$ in a host cell. We assume for the sake of simplicity that genes or DNAs including DNA plasmids are duplicated before cell division.

Assume the period of a cell division cycle to be τ. For instance, in what follows, we use the following piecewise function to describe $v(t)$.

$$v(t)=\begin{cases} e^{(t/\tau-k)\ln 2}, & k\tau \leqslant t < (k+1)\tau \\ 1, & t=(k+1)\tau, \end{cases} \tag{3}$$

where $k=0,1,2,\ldots$. The cell volume factor v exponentially increases from 1 to 2 during each cell cycle, and returns to 1 after cell division.

On the other hand, since DNA is duplicated in a much faster manner than cell volume, we adopt the following sigmoidal function to approximately describe the DNA number factor $u(t)$.

$$u(t)=\begin{cases} ag(t/\tau-k)+b, & k\tau \leqslant t < (k+1)\tau \\ 1, & t=(k+1)\tau, \end{cases} \tag{4}$$

where $k=0,1,2,\ldots$ and $g(t)=1/(1+e^{-\gamma(t-t_d)})$ which is centered at t_d for $0\leqslant t_d\leqslant 1$. $a=1/(g(1)-g(0))$ and $b=1-g(0)/(g(1)-g(0))$ are chosen to ensure $u=1$ and $u=2$ just after and before division, respectively. Clearly the DNA number factor u is mainly constant except the time period near $k\tau+t_d\tau$ during which u rapidly increases from 1 to 2, and returns to 1 after cell division. The period near $k\tau+t_d\tau$ corresponds to the S phase in a eukaryotic cell. In this paper, $t_d=0.6$ and $\gamma=50$ are used to describe $u(t)$. Figure 1 shows $v(t)$ and $u(t)$, where the DNA duplication occurs around $k\tau+t_d\tau=k\tau+0.6\tau=k+0.6$ for each cell cycle period $\tau=1$.

Define $[m(t)]=m(t)/v(t)$ and $[p(t)]=p(t)/v(t)$ as *relative concentrations* of mRNAs and proteins. In the following, the time t for variables $[m]$ and $[p]$ is dropped for simplicity.

Then by differentiating $m(t)=v(t)[m]$ and $p(t)=v(t)[p]$ with respect to t, and further by substituting into Eqs. (1) and (2) with consideration of $v\rightarrow v/2$, $u\rightarrow u/2$, $m\rightarrow m/2$, and $p\rightarrow p/2$ at division, we obtain a model of the gene regulatory network in terms of relative concentrations in a closed form.

$$[\dot{m}]=\frac{u(t)}{v(t)}Nf([p])-(K_m+\overline{v})[m], \tag{5}$$

$$[\dot{p}]=S_p[m]-(K_p+\overline{v})[p], \tag{6}$$

where $\overline{v}=\dot{v}(t)/v(t)=(\ln 2)/\tau$ when $v(t)$ of Eq. (3) is adopted. Note that there is only one factor V_0 between concentration and relative concentration, i.e., the concentration of x is $[x]/V_0$.

Remark II.1. When there is no cell division cycle dynamics, the last terms $(\overline{v}[m],\overline{v}[p])$ in Eqs. (5) and (6) disappear and $v(t)=u(t)=1$.

Clearly, the gene regulatory network of Eqs. (5) and (6) is not an autonomous system due to $u(t)/v(t)$, which generally is a periodic function.

We consider the following two situations for the numbers of genes, i.e., $Nu(t)$ in this paper.

Assumption II.2. (1) Rapid Change: $u(t)$ is constant during the cell growth except the S phase in which $u(t)$ is doubled, but immediately halves after division in each daughter cell, as indicated in Eq. (4). (2) Smooth Change: $u(t)$ proportionally increases with the cell volume growth until it is doubled at the division instant but immediately halves after division in each daughter cell, i.e., $u(t)/v(t)=1$.

1 of Assumption II.2 may correspond to the situation of a eukaryotic cell, whereas 2 of Assumption II.2 is an approximation to a prokaryotic cell with a large number of plasmids. However, 1 of Assumption II.2 may also hold for a system with chromosomal genes in a prokaryotic cell. We assume that all chemicals including DNAs and proteins in a mother cell are equally distributed to two daughter cells after division although many factors, such as stochastic noise, influence the ratio of the distribution, in particular for non-DNA molecular species [22].

B. Dynamics with rapid change of gene numbers

We assume that 1 of Assumption II.2 holds. Such an assumption mainly corresponds to a eukaryotic cell. $u(t)$ is constant during the cell growth, is duplicated in the S phase, and immediately halves after division in each daughter cell, as described by Eq. (4) and Fig. 1. Therefore, by Eqs. (1)–(4) with consideration of $m\rightarrow m/2$, $p\rightarrow p/2$, $v\rightarrow v/2$, and $u\rightarrow u/2$ at the division, we can describe the dynamics in terms of the chemical numbers and v,u by IDEs in a closed form.

$$\dot{m}=Nuf\left(\frac{p}{v}\right)-K_m m-\frac{m}{2}\sum_{k=1}^{\infty}\delta(t-k\tau), \tag{7}$$

$$\dot{p} = S_p m - K_p p - \frac{p}{2}\sum_{k=1}^{\infty} \delta(t - k\tau), \tag{8}$$

$$\dot{v} = \overline{v}v - \frac{v}{2}\sum_{k=1}^{\infty} \delta(t - k\tau), \tag{9}$$

$$\dot{u} = \frac{\gamma}{\tau}(u - b) - \frac{\gamma}{a\tau}(u - b)^2 - \frac{u}{2}\sum_{k=1}^{\infty} \delta(t - k\tau), \tag{10}$$

where the impulse function δ is defined as $\delta(t)=0$ when $t \neq 0$, and $\int_{-\infty}^{+\infty} \delta(t)dt=1$. Notice $v(0)=u(0)=1$. Due to the last terms of Eqs. (7)–(10), values of $m(t)$, $p(t)$, $v(t)$, and $u(t)$ all halve at $t=k\tau$. Clearly Eqs. (7)–(10) are not ODEs but IDEs with a periodic impulse force. The effects of a cell division cycle on the chemical numbers include two parts, i.e., a variable term (v,u) to influence the synthesis of mRNAs, and an impulse term $\delta(t-k\tau)$ to enhance the degradation or dilution of each chemical. As indicated in Eqs. (7)–(10), a cell division cycle may play a significant role in gene regulation due to the nonlinear relation among the cell volume, the numbers of genes, and the gene regulatory network although gene expressions are tightly controlled by transcriptional factors.

Dynamics can also be expressed by Eqs. (5) and (6) in terms of relative concentrations, i.e., by simply substituting Eqs. (3) and (4) into Eqs. (5) and (6). Clearly the effect of a cell division cycle on relative concentrations is a periodic term multiplying with the synthesis rate. Both $u(t)/v(t)$ in Eq. (5) and $\delta(t-k\tau)$ in Eqs. (7)–(10) can be viewed as external forces.

We call Eqs. (3)–(6) or Eqs. (7)–(10) a forced system, and an autonomous system if $v(t)=u(t)=1$ for all t. The oscillation in an autonomous system is also called natural oscillation or internal oscillation. Usually, gene replication and cell division cause the protein numbers to tend to a limit cycle, but depending on the natural oscillation period, there may exist quasiperiodic, resonance, or even chaotic dynamics [32].

C. Dynamics with smooth change of gene numbers

When 2 of Assumption II.2 holds, $u(t)$ proportionally increases with the cell volume, i.e., $u(t)/v(t)=1$. Such an assumption is actually valid only when N is sufficiently large, e.g., with a large number of plasmids in a bacterial cell. Otherwise, $u(t)/v(t)$ should be considered as a time varying number and rapidly change its value as indicated in 1 of Assumption II.2.

Therefore, by Eqs. (5) and (6), we can describe the dynamics in terms of relative concentrations.

$$[\dot{m}] = Nf([p]) - (K_m + \overline{v})[m], \tag{11}$$

$$[\dot{p}] = S_p[m] - (K_p + \overline{v})[p], \tag{12}$$

which are actually autonomous ODEs. The effect of a cell division cycle on relative concentrations is an additional degradation rate $\overline{v}$, which implies that a cell cycle mainly enhances the dilution of chemicals in terms of concentrations or affects gene regulation by acting as a degradation factor.

Notice that the effects of a cell division cycle on the mRNA and protein numbers should be further transformed from the relative concentrations by $m=v(t)[m]$ and $p=v(t)[p]$, e.g., the number $m(t)$ periodically varies with t due to the periodic function v even if $[m]$ is a nonzero constant.

In a real cell, there actually exist many perturbations, such as noises, around $u(t)/v(t)=1$, which prevent the perfect tune of the DNA duplication with cell size, or even equal distribution of DNAs in daughter cells. For the case of deterministic perturbations, i.e., $u(t)/v(t)=1+\sigma$ where σ is a small real number, the stability analysis of equilibria is relatively easy and can be investigated by perturbing N of Eq. (11) due to $Nu(t)/v(t)=N+\sigma N$ according to Eqs. (5) and (6), which can be examined by the Jacobian matrix in the next section.

Next, we examine the effects of stochastic perturbations on the cellular dynamics by using a small stochastic noise model. Let $u(t)/v(t)=1+\sigma\eta(t)$ where σ is a small real number corresponding to the deviation of the small noise, and $\eta(t)$ is Gaussian noise with zero mean $\langle\eta(t)\rangle=0$ and variance $\langle\eta(t)\eta(t')\rangle=\delta(t-t')$. Then, Eqs. (11) and (12) become

$$[\dot{m}] = Nf([p]) - (K_m + \overline{v})[m] + Nf([p])\sigma\eta(t), \tag{13}$$

$$[\dot{p}] = S_p[m] - (K_p + \overline{v})[p]. \tag{14}$$

Clearly, fluctuations of such noise mainly influence the system through the transcription process due to Eq. (13). We will examine Eqs. (13) and (14) for the effects of the stochastic noise by the numerical simulation.

D. Stability of periodic oscillation

Local stability analysis of dynamics of Eqs. (11) and (12) at an equilibrium point for $[m]$ and $[p]$ is straightforward, by simply investigating the eigenvalues of the Jacobian matrix J for Eqs. (11) and (12), i.e.,

$$J = \begin{bmatrix} -K_m - \overline{v}, & Ndf([p])/d[p] \\ S_p, & -K_p - \overline{v} \end{bmatrix}. \tag{15}$$

Notice that stability of $[m]$ and $[p]$ for an equilibrium point is identical to that of m and p for the corresponding periodic solution. By including nonlinear terms, we can also analyze local bifurcations [33].

Next, we examine the stability of a periodic oscillation for Eqs. (7)–(10). For the sake of simplicity, Eqs. (7)–(10) are summarized as

$$\dot{X}(t) = F(X(t)) - \frac{1}{2}\sum_{k=1}^{\infty} \delta(t - k\tau)X(t), \tag{16}$$

where $X=(m,p,v,u)$, and $F=F(m,p,v,u)=(Nuf(p/v) - K_m m, S_p m - K_p p, \overline{v}v, \gamma(u-b)/\tau - \gamma(u-b)^2/(a\tau))$.

Let $\phi(t;X(k\tau))$ denote the flow of the vector field F starting from $\phi(0;X(k\tau))=X(k\tau)$ at $t=0$, i.e.,

$$\frac{d\phi(t;X(k\tau))}{dt} = F(\phi(t;X(k\tau))). \tag{17}$$

Define $\psi(t)$ as a fundamental solution satisfying

$$\frac{\partial \psi(t)}{\partial t} = \frac{\partial F(\phi(t;X(k\tau)))}{\partial X}\psi(t) \tag{18}$$

with $\psi(0)=E$, where $\psi \in R^{(2n+2)\times(2n+2)}$, and E is an identity matrix.

Then integrating Eq. (16) from $k\tau^+$ to t for $k\tau < t < (k+1)\tau$ yields

$$\begin{aligned} X(t) - X(k\tau^+) &= \int_{k\tau^+}^{t} F(X(t))dt \\ &= \int_{0}^{t-k\tau} F(\phi(t;X(k\tau)))dt \\ &= \phi(t-k\tau;X(k\tau)) - \phi(0,X(k\tau)) \end{aligned} \tag{19}$$

according to Eq. (17). Notice that $X(k\tau^+)=\phi(0,X(k\tau))$, and the integration range is changed for $\phi(t,X(k\tau))$ in Eq. (19) due to its initial state starting from $X(k\tau)$. On the other hand, in the same way, by integrating Eq. (16) from $k\tau^+$ to $(k+1)\tau$ for t, we have

$$\begin{aligned} X((k+1)\tau) - X(k\tau^+) &= \int_{0}^{\tau} F(\phi(t;X(k\tau)))dt \\ &\quad - \frac{1}{2}\int_{0}^{\tau} \delta(t-\tau)\phi(t,X(k\tau))dt \\ &= \phi(\tau;X(k\tau)) - \phi(0,X(k\tau)) \\ &\quad - \frac{1}{2}\phi(\tau;X(k\tau)). \end{aligned} \tag{20}$$

Therefore by using ϕ of the autonomous system and from Eqs. (19) and (20), the orbit of the nonautonomous Eq. (16) can be expressed with $k=0,1,2,\ldots,$

$$X(t) = \phi(t-k\tau,X(k\tau)), \quad k\tau \leqslant t < (k+1)\tau, \tag{21}$$

$$X((k+1)\tau) = \frac{1}{2}\phi(\tau,X(k\tau)), \quad t=(k+1)\tau. \tag{22}$$

Clearly, different from the continuous dynamics of the concentrations, e.g., Eqs. (11) and (12), the chemical number $X(t)$ is continuous at $t=k\tau$ from the right side but generally discontinuous at $t=k\tau$ from the left side. Equation (22) is a Poincaré map of Eq. (16).

Thus the necessary and sufficient condition of a period-one solution of Eq. (16) is the existence of a real solution for the following equation:

$$X(k\tau) = \frac{1}{2}\phi(\tau,X(k\tau)). \tag{23}$$

Notice that ϕ is not the flow of the right-hand-side of Eq. (16) but the flow of the vector field F of Eq. (17).

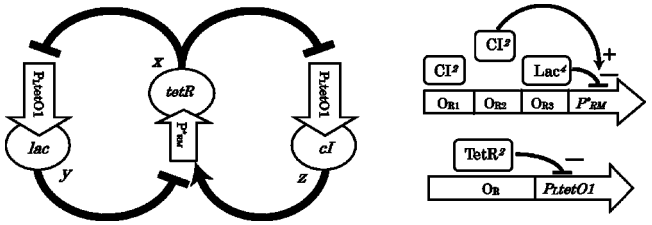

FIG. 2. A three-gene model of a gene regulatory network (protein Lac forms a tetramer to inhibit gene-*tetR*, and protein CI enhances gene-*tetR* as a dimer, whereas protein TetR forms a dimer to repress both gene-*lac* and gene-*cI*). P^*_{RM} is a mutated promoter of P_{RM} and has two binding sites (O_{R1} and O_{R2}) for protein dimer CI^2 and one binding site (O_{R3}) for protein tetramer Lac^4. Affinities of CI^2 for P^*_{RM} are $O_{R1} > O_{R2}$. Binding effects of CI^2 to O_{R1} and O_{R2} for transcription of P^*_{RM} are neutral and positive, respectively, in contrast to a negative binding effect of O_{R3} by Lac^4. On the other hand, there is one binding site, i.e., O_R for protein $TetR^2$, which represses the transcription of the promoter $P_L tetO1$. x: number of protein TetR; y: number of protein Lac; and z: number of protein CI.

According to Eq. (22), the stability of the period-one solution depends on the eigenvalues of the Jacobian matrix at $X(k\tau)$:

$$J = \frac{1}{2}\frac{\partial \phi(\tau,X(k\tau))}{\partial X(k\tau)} = \frac{1}{2}\psi(\tau). \tag{24}$$

From dynamical system theory, if absolute values of eigenvalues for J are all less than 1, then the periodic solution is asymptotically stable [33]. In a similar manner, we can derive the existence and stability conditions for any period-k solution.

III. IMPLEMENTATION OF A THREE-GENE NETWORK EXAMPLE

We design a network by genes *lac*, *tetR*, and *cI* with the promoters $P_L tetO1$, P^*_{RM}, and $P_L tetO1$, shown in Fig. 2. All three genes are well-characterized prokaryotic transcriptional regulators, which can be found in bacterium *E. coli* and λ phage. Protein Lac forms a tetramer to inhibit gene-*tetR* with promoter P^*_{RM} and protein CI activates the gene-*tetR* as a dimer, whereas protein TetR forms a homodimer to repress both gene-*lac* and gene-*cI* with promoters $P_L tetO1$. All three genes can be engineered on plasmids, and can then be cloned to multiple copies, e.g., by PCR. The engineered plasmids are further assumed to grow in *E. coli*.

Let x, y, and z be the numbers of protein monomers TetR, Lac, and CI, respectively. Then for gene-*cI* with promoter $P_L tetO1$, define x_2 to be the number of protein dimer TetR. Let d and d_{x_2} be the number of free DNA and the number of the $TetR^2$-DNA complex, i.e., O_R bound by a protein dimer $TetR^2$.

Then for gene-*cI*, the multimerization and binding reactions can be written as the equilibrium reactions [9]

$$x + x \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} x_2; \quad d + x_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} d_{x_2}. \tag{25}$$

The basic mechanism of chemical reactions is stochastic collisions of chemicals according to the law of mass action [29]. Therefore the reaction of Eq. (25) can be expressed by differential equations,

$$\dot{x}_2 = V\left(k_1\frac{x^2}{V^2} - k_{-1}\frac{x_2}{V}\right); \quad \dot{d}_{x_2} = V\left(k_2\frac{x_2 d}{V^2} - k_{-2}\frac{d_{x_2}}{V}\right).$$

Thus the equilibrium reactions of fast dynamics Eq. (25) can be written as algebraic equations [9]: $x_2 = k_1 x^2/(k_{-1}V) = c_1 x^2/v$; $d_{x_2} = k_2 x_2 d/(k_{-2}V) = \sigma_3 x^2 d/v^2$, where $\sigma_3 = c_1 c_2$ and $c_i = k_i/(k_{-i}V_0)$. Such algebraic equations imply that the numbers of the chemicals synthesized in the fast dynamics are inversely proportional to the cell volume.

Let the copy number of plasmids with gene-*cI* be $n_z u(t)$. Then we have a conservation condition: $n_z u(t) = d + d_{x_2}$, which leads to $d = n_z u(t)/(1+\sigma_3 x^2/v^2)$. Therefore by substituting the equilibrium equations of fast dynamics, the slow dynamics for mRNA and the synthesized protein representing the transcription and translation processes of gene-*cI* are

$$\dot{m}_z = \beta_{m_z} d - k_{m_z} m_z = \frac{\beta_{m_z} n_z u(t)}{1+\sigma_3 x^2/v^2} - k_{m_z} m_z, \tag{26}$$

$$\dot{z} = s_z m_z - k_z z, \tag{27}$$

where the synthesis rate of m_z is $\beta_{m_z} d$ due to the repressive effect of TetR on the binding site O_R as indicated in Fig. 2. $x \to x/2$, $y \to y/2$, $z \to z/2$, $v \to v/2$, and $u \to u/2$, at division.

As the same way as the derivation of the gene-*cI*, we can get the dynamics for genes *lac* and *tetR* [8,9]. By defining the relative concentrations for proteins as $[x] = x/v$, $[y] = y/v$, and $[z] = z/v$, the dynamical system of the three-gene network is summarized in terms of the relative concentrations of proteins in the following closed form:

$$[\dot{m}_x] = \beta_{m_x} n_x \frac{u(t)}{v(t)} f_x([y],[z]) - (k_{m_x} + \overline{v})[m_x], \tag{28}$$

$$[\dot{x}] = s_x[m_x] - (k_x + \overline{v})[x], \tag{29}$$

$$[\dot{m}_y] = \beta_{m_y} n_y \frac{u(t)}{v(t)} f_y([x]) - (k_{m_y} + \overline{v})[m_y], \tag{30}$$

$$[\dot{y}] = s_y[m_y] - (k_y + \overline{v})[y], \tag{31}$$

$$[\dot{m}_z] = \beta_{m_z} n_z \frac{u(t)}{v(t)} f_z([x]) - (k_{m_z} + \overline{v})[m_z], \tag{32}$$

$$[\dot{z}] = s_z[m_z] - (k_z + \overline{v})[z], \tag{33}$$

where $f_x([y],[z]) = (1 + c[z]^2 + \alpha\sigma_1 c^2[z]^4)/((1 + c[z]^2 + \sigma_1 c^2[z]^4)(1+\sigma_4[y]^4))$, $f_y([x]) = 1/(1+\sigma_2[x]^2)$, and $f_z([x]) = 1/(1+\sigma_3[x]^2)$.

The parameters in Eqs. (28)–(32) are set as follows: $\sigma_1 = 2$, $\sigma_2 = 0.25$, $\sigma_3 = 7.5$, $\sigma_4 = 3.5 \times 10^{-4}$, $\beta_{m_x} = 20/3$, $\beta_{m_y} = 100/3$, $\beta_{m_z} = 5/3$, $\alpha = 11$, $k_x = 5000/3$, $k_y = 3000/3$, $k_z = 5/6$, and $c = 0.0165$. At the beginning of cell growth, $n_x = 60$, $n_y = 60$, and $n_z = 60$. $k_{m_i} = 10/3$, $s_i = 100/3$ for $i = x, y$. $k_{m_z} = 1/3$, $s_z = 5/6$. Let the cell cycle be $\tau = 1$ which stands for 30 min. The parameters for Eqs. (28) and (29) are from [8,9] with slight modification, while parameters for Eqs. (30)–(33) are set appropriately in biologically realistic ranges, due to lack of experimental data. Since we want to construct a relaxation oscillator by making use of fast-slow dynamics, the RBS (ribosome-binding site) of gene-*cI* is assumed to be artificially engineered so that the translation efficiency of protein CI is significantly slower than those of proteins Lac and TetR.

Furthermore, the binding affinity of the proteins Lac and TetR to DNA can also be changed by introducing small molecules IPTG (isotropyl-β-D-thiogalactopyranoside) and aTc (anhydrotetracycline), respectively, which bind to tetramer Lac^4 and dimer $TetR^2$ and prevent them from binding to operator sites [9]. In order to measure the behaviors of the genetic network, a gene for GFP (green fluorescent protein) or YFP (yellow fluorescent protein) is assumed to be incorporated in each plasmid under the control of a targeted promoter to monitor the targeted gene in experiments [6].

IV. NUMERICAL EXPERIMENTS

A. Case for smooth change of gene numbers

Assume that 2 of Assumption II.2 holds, i.e., the DNA number and the cell volume increase at an identical rate: $u(t)/v(t) = 1$ in Eqs. (28)–(33).

We first use genes *lac* and *tetR* of Fig. 2 to construct a gene switch, which means that the dynamics of the gene switch are governed by Eqs. (28)–(31) with $[z]$ fixed.

Figure 3 (top figure) shows null-clines for Eqs. (28) and (29) (solid line) and Eqs. (30) and (31) (dotted line) when $[z] = 6$ and $v(t) = u(t) = 1$. There are two stable equilibria and one unstable equilibrium (middle one). Taking $[z]$ as a parameter, it is easy to check that bistable region for $[z]$ is $3.69 \leqslant [z] \leqslant 30.1$ from the analysis of the gene switch. When $[z] < 3.69$, there is only one stable equilibrium with high $[y]$ and low $[x]$. On the other hand, there is also only one stable equilibrium with low $[y]$ and high $[x]$ when $[z] > 30.1$.

The second figure of Fig. 3 indicates the regions of unistability (one stable equilibrium) and bistability (two stable equilibria) with parameters k_{m_x} and k_{m_y}. Solid lines and dotted lines are the boundaries between the unistable and bistable regions without a cell cycle ($v = u = 1, \overline{v} = 0$) and with a cell cycle **(**$v/u = 1, \overline{v} = (\ln 2)/\tau$**)**, respectively. Clearly with and without a cell cycle, dynamics are almost identical for some regions but qualitatively change for other regions. Figure 4 is an example with parameters $k_{m_x} = 5$ and $k_{m_y} = 4.5$ as marked by an asterisk in the second figure of Fig. 3, showing such qualitative changes. In the left two figures, the dynamics without a cell cycle converge to two stable equilibria (bistability) from two different initial conditions. In contrast, the system with a cell cycle loses the bistability and converges to a single equilibrium (unistability) for any initial conditions as demonstrated in the right two figures. The difference with and without a cell cycle for the gene switch is

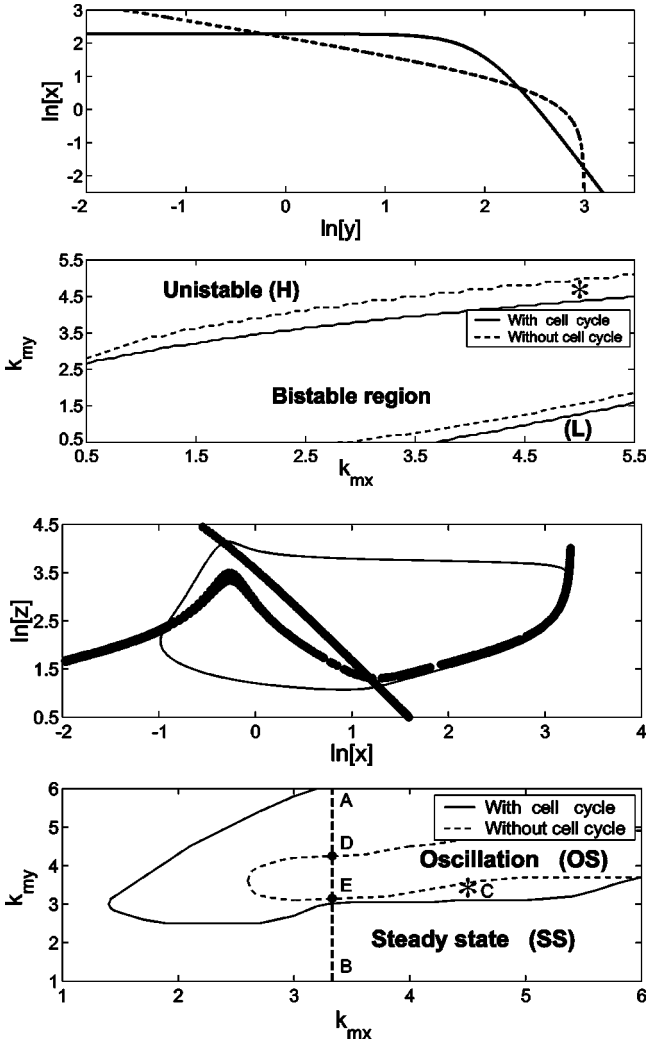

FIG. 3. Null-clines and stability regions for a switch and an oscillator. The top figure is null-clines of switch for Eqs. (28) and (29) (solid line) and Eqs. (30) and (31) (dotted line) when $[z]=6$ and $v(t)=u(t)=1, \overline{v}=0$. The second figure is the unistable (one stable equilibrium) and bistable (two stable equilibria) regions with and without a cell cycle. H and L stand for the high and low stable equilibria, respectively. The third figure is null-clines of an oscillator for Eqs. (28)–(33) and there is a limit cycle on the $[x]-[z]$ plane with $v(t)=u(t)=1$. The lower-right figure is the oscillatory regions (OS) and steady state regions (SS) with and without a cell cycle.

the last terms of Eqs. (28)–(31), which are degradations attributed to a cell cycle. If the original degradation rates are far bigger than $\overline{v}$, the effect of a cell division cycle on the dynamics of the gene switch is not significant. However, when the system is near the stability boundary with respect to the degradation terms or when original degradation rates are comparable to or less than $\overline{v}$, the cell division cycle strongly affects the performance of the system. Note that comparing to mRNAs, proteins usually degradate much slowly.

Next, we show that the network of Fig. 2 or Eqs. (28)–(33) can be used as a relaxation oscillator. Since the translation efficiency of protein CI can be significantly reduced by adjusting the RBS or other manipulations, Eqs. (28)–(31) are fast dynamics and Eqs. (32) and (33) can be slow dynamics.

Figure 3 (the third figure) shows the null-clines of Eqs. (28)–(33), and a limit cycle on a $[x]-[z]$ plane when $v(t)=u(t)=1, \overline{v}=0$, which is a typical representation of a relaxation oscillator. The bottom figure is the boundaries between oscillation (OS) and steady state (SS) with and without a cell cycle. The clear difference for OS regions indicates that a cell cycle may also significantly affect dynamical behaviors of periodic oscillations. Fig. 5 shows such influence of the cell cycle on the relaxation oscillator with parameters $k_{m_x}=4.5$ and $k_{m_y}=3.5$, whose location is shown in the bottom figure of Fig. 3 by an asterisk. The lower figure is the dynamics with a cell cycle, which has a typical relaxation oscillation with the period $T=4.9$. However, without a cell cycle, as indicated in the upper figure, the oscillation disappears and dynamics converge to a steady state.

According to Eqs. (28)–(33), the effect of a cell division cycle on the concentrations can be viewed as an additional degradation rate $\overline{v}$, which mainly reduces the synthesis of each corresponding chemical. Depending on the interaction of each chemical in gene networks, such degradation effects of a cell cycle may significantly affect the dynamics both quantitatively and qualitatively.

Furthermore, assuming imperfect synchronization of u and v, we examine the effects of stochastic perturbations around $u(t)/v(t)=1$ using model Eqs. (13) and (14), i.e., substitute $u(t)/v(t)=1+\sigma\eta(t)$ into Eqs. (28)–(33). Figure 6 shows the simulation results for $\sigma=0.05$ and 0.2 that stochastically perturb the system around a periodic orbit and a stable equilibrium. Clearly, when the perturbation is sufficiently small, e.g., $\sigma=0.05$, there is no qualitative change, the system almost moves along the original trajectories, and the deviations are also quite small, which are indicated by the light vertical lines in the figures. However, when the noise deviations become large, e.g., $\sigma=0.2$, dynamics exhibit wild and unpredictable behaviors due to the strong influence of stochastic noises, which implies that the imperfect synchronization of u and v is also an important factor when designing or modeling genetic networks.

B. Case for rapid change of gene numbers

Assume that 1 of Assumption II.2 holds. Then, Eqs. (28)–(33) can also be expressed as follows:

for $w=x,y,z$

$$\dot{m}_w = \beta_{m_w} n_w u f_w - k_{m_w} m_w - \frac{m_w}{2}\sum_{k=1}^{\infty} \delta(t-k\tau), \qquad (34)$$

$$\dot{w} = s_w m_w - k_w w - \frac{w}{2}\sum_{k=1}^{\infty} \delta(t-k\tau), \qquad (35)$$

where $v(t)$ and $u(t)$ follow Eqs. (9) and (10). In the same manner as the case with smooth change of the gene numbers, we can construct gene switches or oscillators, and further theoretically analyze them. For instance, when we consider the autonomous system, i.e., without a cell division cycle

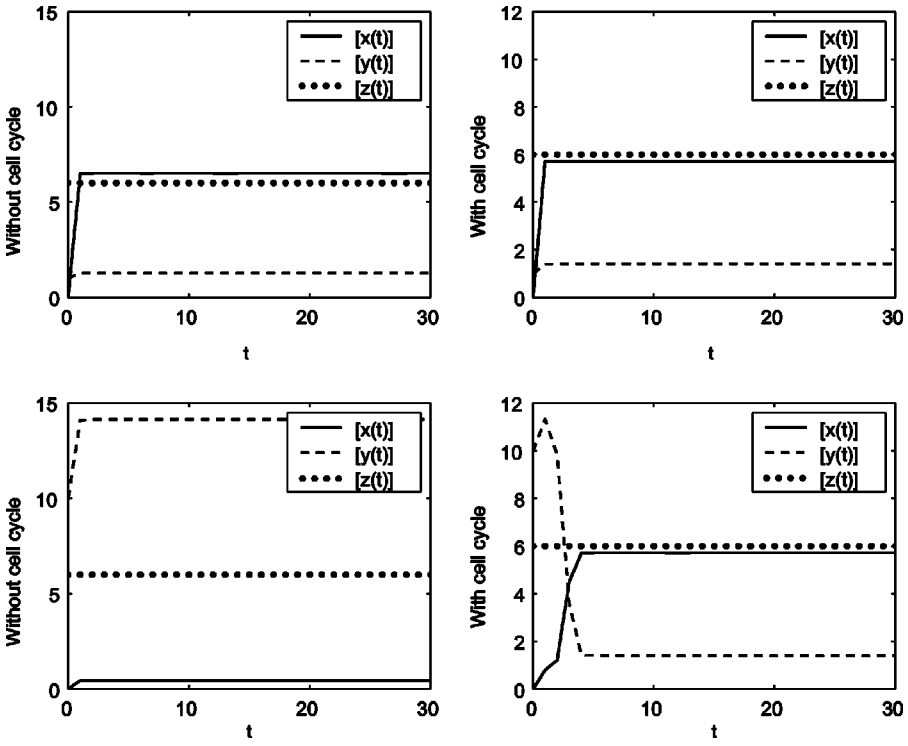

FIG. 4. Time evolutions of a switch with genes *lac* and *tetR* with $[z]=6$ for the case with smooth change of the gene numbers (left half figures are for the case without a cell cycle from two different initial conditions, whereas the right half figures are for the case with a cell cycle from two different initial conditions).

$v(t)=u(t)=1$, the null-clines are the same as Fig. 3. Let the natural period of the oscillation in the autonomous system be T. Theoretically, we can expect synchronization between the natural oscillation with period T and the forced oscillation with period τ for Eqs. (28)–(33), or even resonance if $Ti \simeq j\tau$, where i and j are integers without a common divisor.

Figure 7 is the analysis of the effects for a cell cycle by parameters k_{m_y}, t_d, and τ. The upper figure is the bifurcation diagram without a cell cycle, which shows two Hopf bifurcations corresponding to the lower and upper boundaries in the bottom Fig. 3 at $k_{m_x}=10/3$. Theoretically, due to mechanism of the relaxation oscillator in Fig. 3, the Hopf bifurcations are singularly homoclinic provided that the time scale difference between fast and slow dynamics is sufficiently large [13]. Such a fact implies that the amplitude increases or decreases almost exponentially near the bifurcation points, which is shown in the upper figure. The middle and lower figures are the effects of t_d and τ on amplitudes with a cell cycle. The natural period is $T=23.4$. From the observation of the oscillation amplitude against the cell cycle period τ, there is no noticeable resonance due to extremely strong entrainment of the cell cycle dynamics, which is quite different from the results of [9]. Besides the different networks and cell cycle models, one reason for such a difference is that the cell cycle dynamics $v(t)$ and $u(t)$ in this paper are nonlinearly coupled with the gene network as shown in Eqs. (28)–(33) in contrast to the linear coupling in [9]. Actually the effect of the cell cycle mainly dilutes the concentrations of the chemicals by acting as a degradation factor, which usually reduces the chemical amplitude even at resonance, comparing with the case without a cell cycle. The trend is that the amplitude slowly increases with the cell cycle τ although it is rugged. Generally, the protein synthesis can be enhanced if the DNA duplication occurs at the early stage of the cell cycle, i.e., t_d is small. However, if there are nonlinear interactions among genes in the network, relations can be very complicated. The middle figure is the analysis of the oscillation amplitude variation with t_d that controls the DNA synthesis process. For small t_d, oscillations of x have very small amplitudes, which are caused by strong repression of y due to the early DNA duplication of y. When t_d is around 0.4, there is a significant increase for the protein $[x]$, which is owing to the strong and dominant activation of z. Most of the oscillations are periodic in the middle and lower figures, which are different from the Repressilator shown in the next case.

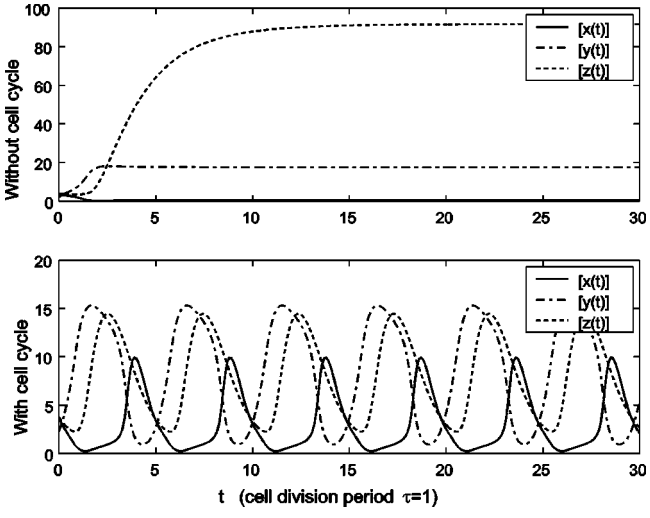

FIG. 5. Time evolutions of the three-gene network without and with a cell division cycle for the case with smooth change of the gene numbers. (The upper figure is the case without a cell division cycle, and the lower figure is the case with a cell division cycle, respectively, at $k_{m_x}=4.5$ and $k_{m_y}=3.5$.)

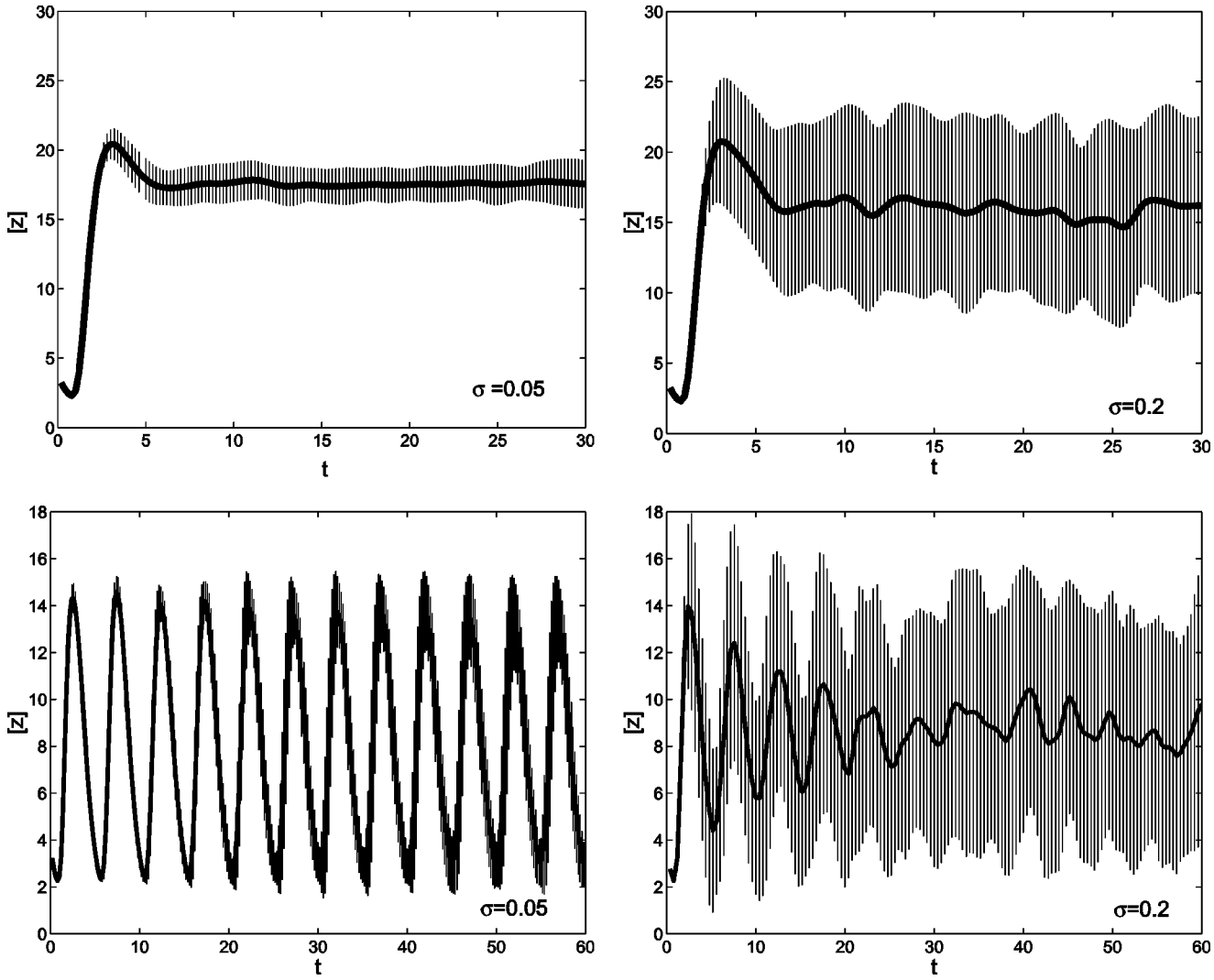

FIG. 6. Time evolutions of the three-gene network with stochastic perturbations for $\sigma=0.05$ and 0.2. (The bold lines are mean values and the light vertical lines represent deviations. The upper figures are the perturbation cases of a stable equilibrium at $k_{m_x}=4.5$ and $k_{m_y}=3$. The lower figures are the perturbation cases of an oscillator at $k_{m_x}=4.5$ and $k_{m_y}=3.5$.)

Figure 8 shows time evolutions for a case without a cell cycle and two cases with the cell cycle corresponding to $t_d=0.38$ and $t_d=0.6$, respectively, where the cell cycle period and the natural period are $\tau=1$ and $T=23.4$. The dynamics are all entrained to be a forced oscillation with a period $\tau=1$ or its subharmonics when a cell cycle is considered, which implies that a cell cycle has a tendency to entrain subsystems of the cell. Comparing with the case of $t_d=0.6$, the synthesis of the protein $[x]$ for the case of $t_d=0.38$ is considerably inhibited by strong repression of Lac *y* due to its early DNA duplication. However, comparing with the case without a cell cycle, the proteins for the cases with a cell cycle are significantly reduced due to the dilution or degradation effect of the cell division as well as the interactions from *y* and *z*.

C. Case for Repressilator

We next examine the influence of the cell cycle on the gene regulation of the Repressilator, whose periodic oscillation was experimentally investigated in *Escherichia coli in vivo* [6]. For the case with cell cycle dynamics, the model can be expressed as follows [6,12]:

$$[\dot{m}_i] = \frac{u}{v}\frac{\alpha}{1+[p_j]^n} - (\alpha_1+\overline{v})[m_i], \tag{36}$$

$$[\dot{p}_i] = \beta[m_i] - (\beta+\overline{v})[p_i], \tag{37}$$

where i and j have the following three pairs of values: $(i=1, j=2)$, $(i=2, j=3)$, and $(i=3, j=1)$, which represent *lacI*, *tetR*, and *cI* respectively. $v(t)$ and $u(t)$ follow Eqs. (9) and (10). n is the Hill coefficient. $[m_i] \in \mathcal{R}$ are the relative concentrations of mRNAs, and $[p_i] \in \mathcal{R}$ are those of proteins. Parameters are set as $n=2$, $\beta=0.5$, $\alpha_1=1$, and $\alpha=500$. Let the cell cycle period $\tau=1$. Assume that there are a small number of plasmid copies in the cell. Then the model can also be described by the IDE form of Eqs. (7)–(10) in terms of the chemical numbers.

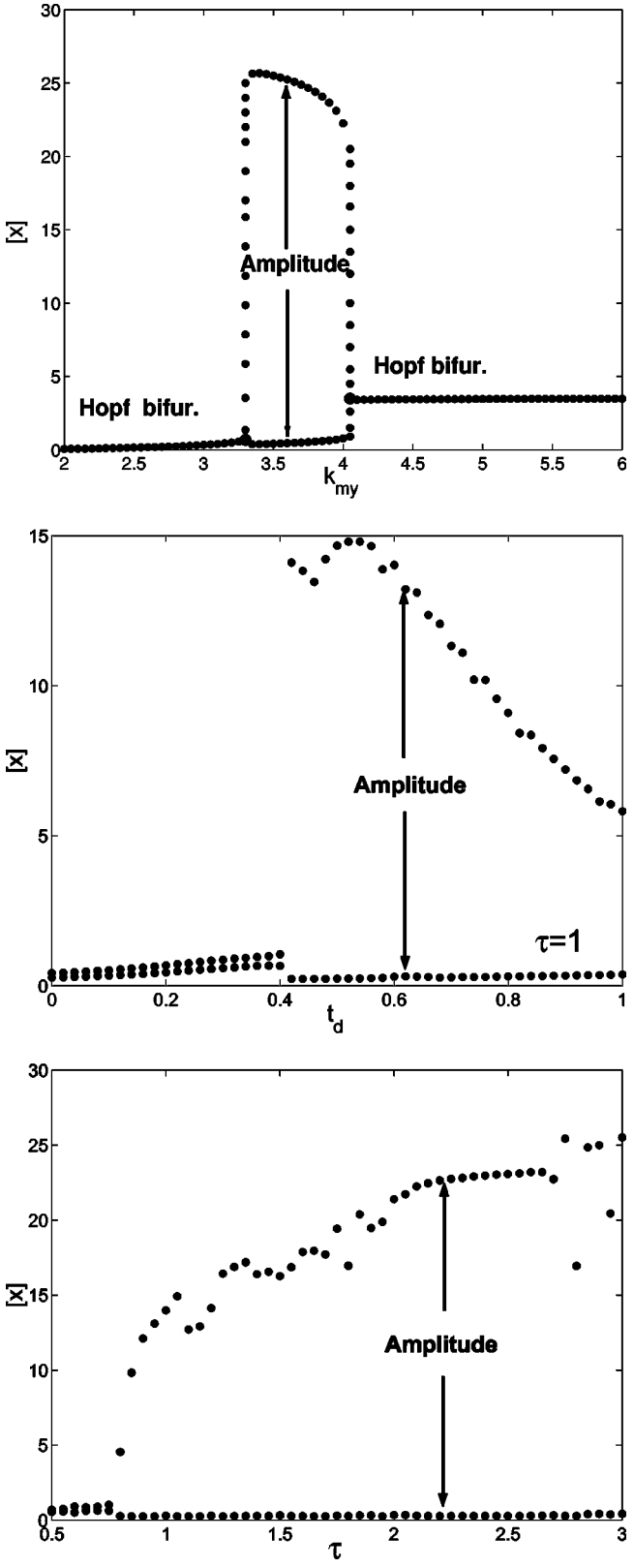

FIG. 7. Analysis of the effect for a cell cycle by parameters k_{m_x}, t_d, and τ. (The upper figure is the bifurcation diagram without a cell cycle, whereas the middle and lower figures are the effects of t_d and τ on the amplitudes with a cell cycle.)

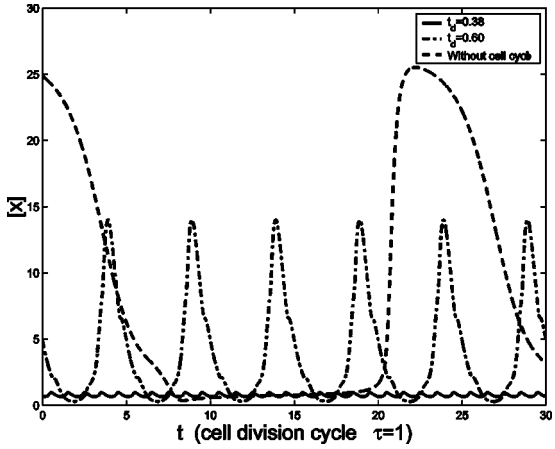

FIG. 8. Time evolutions of the three-gene network for a case without a cell cycle and two cases with the cell cycle corresponding to $t_d=0.38$ and $t_d=0.6$, respectively, when the gene numbers are rapidly changed. (The cell cycle period and the natural period are $\tau=1$ and $T=23.4$.)

Figure 9 is the analysis of the effects of a cell cycle by parameters α, β, t_d, and τ. The upper figure shows the stable and unstable state regions for a case without a cell cycle ($u=v=1$, $\bar{v}=0$) and a case with a smooth change cell cycle **(**$u/v=1$, $\bar{v}=(\ln 2)/\tau$**)**. When α is small, there is a significant difference for the regions, for which the system with a cell cycle has a small oscillation region. The middle and lower figures are the effects of t_d and τ on the amplitude of p_1 with a rapid change cell cycle **(**v and u follow Eqs. (9) and (10), and $\bar{v}=(\ln 2)/\tau$**)**. The trend for the effect of t_d is very clear. The earlier the DNA duplicates, the more the proteins are synthesized. The natural period is $T=27.6$ for the current parameter setting. In contrast, the maximum amplitude increases with the cell cycle τ. Due to the coupling of the natural and forced oscillations, there may exist aperiodic or chaotic attractors. Figure 10 is an example with $\tau=1$, which shows the coexistence of a chaotic attractor with a positive Lyapunov exponent, and a quasi-periodic attractor. Such complex dynamics provide the suitable blend of stability and flexibility needed by the system, and may greatly enrich the rhythms of living organisms to facilitate sophisticated functions, in particular under uncertain environments. Moreover, the irregular behaviors of living organisms may be attributed not to stochastic noise but to the deterministic mechanism.

As the same as the network of Fig. 2, there is no drastic change between resonance and no-resonance cases in terms of amplitudes of oscillations due to extremely strong entrainment of the cell cycle dynamics that nonlinearly exerts on the gene network.

V. CONCLUSION

We have presented a theoretical framework to model and analyze the nonlinear dynamics of gene regulatory networks with the consideration of a cell division cycle, in particular for switches and oscillators of synthetic networks. Specifically, under the assumptions, we have derived two models that may correspond to the eukaryotic and prokaryotic cells,

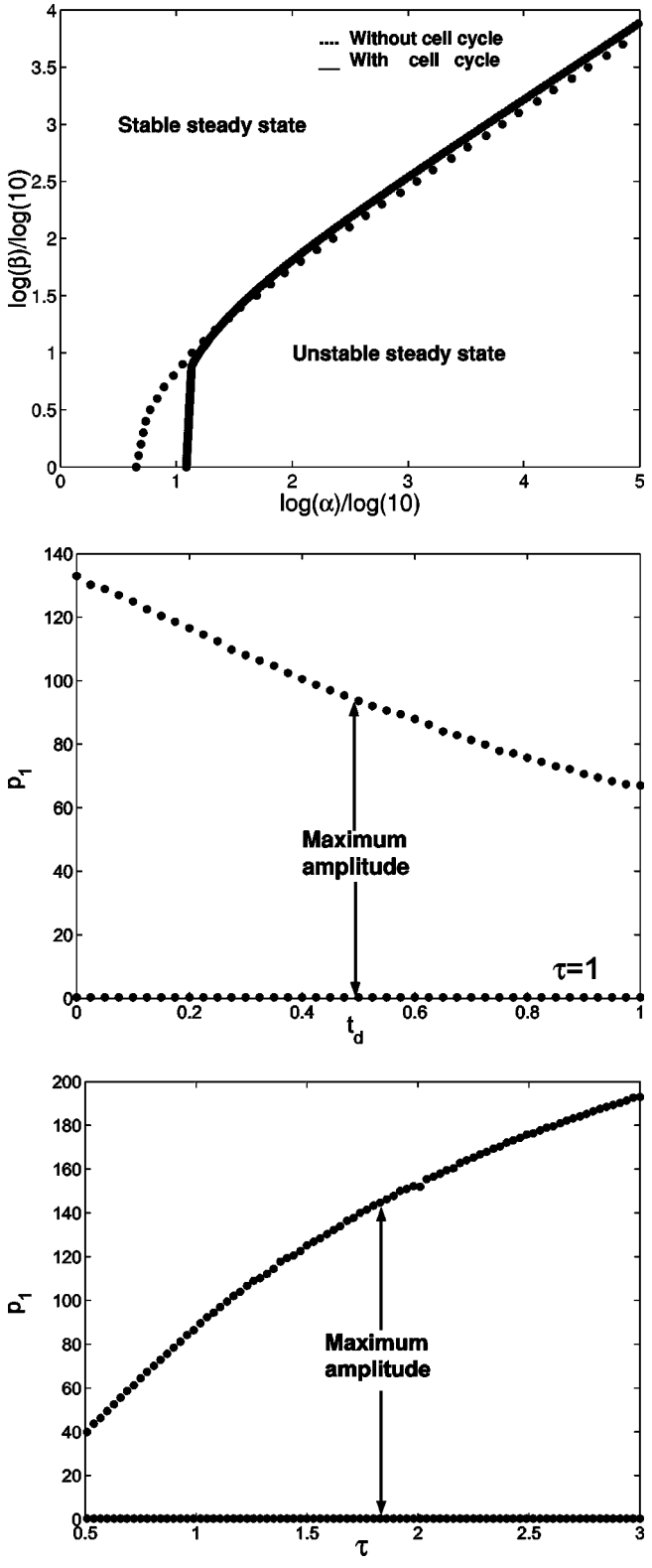

FIG. 9. Analysis on Repressilator. The upper figure shows the stable and unstable state regions for a case without a cell cycle ($u=v=1$, $\overline{v}=0$) and a case with a smooth change cell cycle [$u/v=1$, $\overline{v}=(\ln 2)/\tau$]. The middle and lower figures are the effects of t_d and τ on amplitude of p_1 with a rapid change cell cycle [v and u follow Eqs. (9) and (10), and $\overline{v}=(\ln 2)/\tau$].

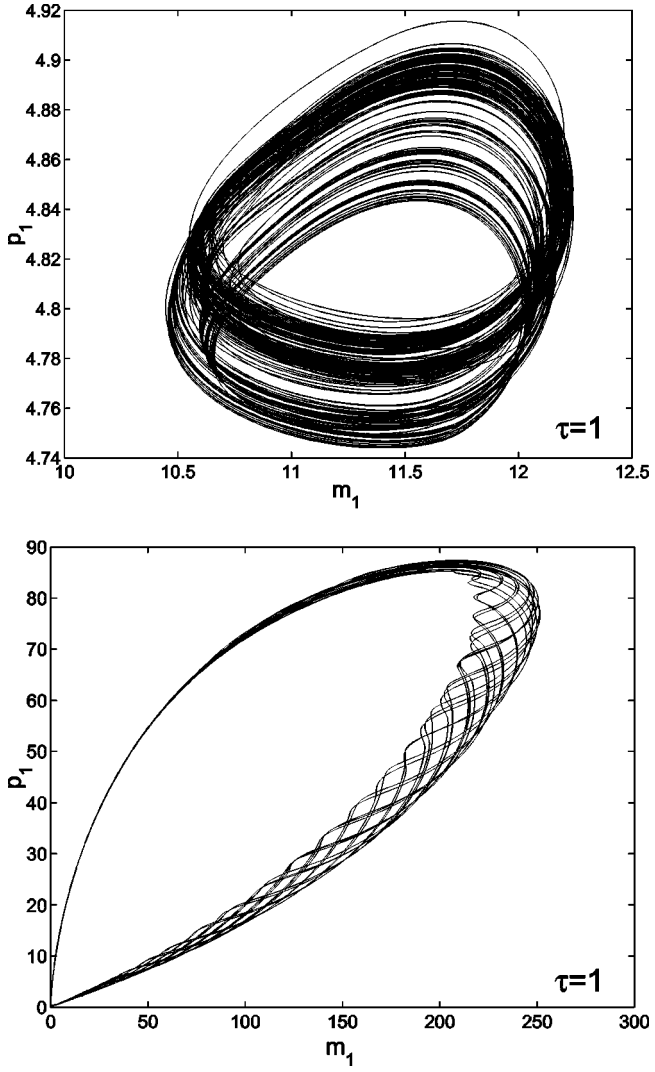

FIG. 10. Coexistence of a chaotic attractor (the top figure) and a quasi-periodic attractor (the bottom figure) with two different initial conditions for Repressilator ($\tau=1$ and $t_d=0.6$).

respectively. For the case with the smooth change of the gene numbers, the influence of a cell division cycle can be modeled as an additional degradation rate for each chemical when the concentrations are concerned, whereas the cell dynamics for the chemical numbers in gene regulatory networks can be expressed by IDEs with periodic degradation terms for the case with the rapid change of the gene numbers.

In particular, when the gene network is near a stability boundary or when the original degradation rates of the network are small, a cell cycle as a degradation factor may significantly affect cellular dynamics both qualitatively and quantitatively.

We have used a three-gene model (*lac*, *tetR*, *cI*) and the Repressilator as switch and oscillator examples to illustrate our theoretical results.

As indicated in this paper, for the case with a smooth change of the gene numbers, an effect of the cell division cycle on gene regulation mainly accelerates the degradation of the chemical concentrations. Depending on the structure of the gene network, such an effect may change dynamics

both quantitatively and qualitatively, as shown in the numerical simulation.

For a dynamical gene switch, the bistable region may disappear due to cell division dynamics although there is a bistable region for the autonomous system, and vice versa.

For a genetic oscillator, a cell division cycle works as an external force to entrain or synchronize the natural oscillation.

Usually, a cell cycle entrains the system to tend to a limit cycle, but depending on the natural oscillation period or network structure, there may exist quasi-periodic, resonance or even chaotic dynamics, stimulated by the cell cycle.

A genetic network in vivo in a cell and an artificial genetic network in vitro in a cell-free system [34] actually correspond our model with and without a cell division cycle, respectively. Therefore such analyses with and without a cell division cycle may be a theoretical basis to quantitatively predict the essential dynamics and to successfully implement experiments from *in vitro* to *in vivo*.

Due to a cell division cycle, there is a wide range distribution in terms of the molecule numbers of proteins at an equilibrium of the concentrations even without noise. In other words, a cell division cycle not only is an important part of dynamics but also can be viewed as a major source of fluctuation for gene networks, which affects both gene expression levels and stability of cellular dynamics.

A cell cycle is viewed as an external force to drive autonomous cellular dynamics in this paper. Such a technique or mechanism can be also applied to analyze effects of a circadian oscillation on gene networks, e.g., cellular dynamics of cyanobacteria, where surprisingly almost all of genes are controlled by the circadian clock [35]. In contrast to many other living organisms, with and without the periodic clock or external force, cyanobacteria may have qualitatively different behaviors, which imply that the gene regulation mechanism may be quite different from others.

In addition, depending on the organisms or cells, there are different cell cycles, which may be important factors for the formation of cell specificity, because the cell cycles influence entire protein dynamics and differentiation of the cell.

In this paper, although we have mainly examined effects of a cell cycle on the cellular dynamics, there are also other important factors, which may play crucial roles in biological processes and should be further investigated in future works from both theoretical and experimental viewpoints, such as stochastic noise [20,22,28] and time delays [12,13,19]. In addition, the cell cycle model used in this paper should be further improved to reflect various rhythms in living organisms. It is also necessary to examine the features induced by a cell cycle and their physiological relevance in the future.

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[1] A. Keller, J. Theor. Biol. **172**, 169 (1995).
[2] P. Smolen, D. A. Baxter, and J. H. Byrne, Neuron **26**, 567 (2000).
[3] D. M. Wolf, and F. H. Eeckman, J. Theor. Biol. **195**, 167 (1998).
[4] T. Mestl, E. Plahte, and S. W. Omholt, J. Theor. Biol. **176**, 291 (1995).
[5] T. S. Gardner, C. R. Cantor, and J. J. Collins, Nature (London) **403**, 339 (2000).
[6] M. B. Elowitz and S. Leibler, Nature (London) **403**, 339 (2000).
[7] A. Becskei and L. Serrano, Nature (London) **405**, 590 (2000).
[8] J. Hasty, F. Isaacs, M. Dolnik, D. McMillen, and J. J. Colins, Chaos **11**, 207 (2001).
[9] J. Hasty, M. Dolnik, V. Rottschafer, and J. J. Collins, Proc. Natl. Acad. Sci. U.S.A. **88**, 148101 (2002).
[10] D. McMillen, N. Kopell, J. Hasty, and J. J. Collins, Proc. Natl. Acad. Sci. U.S.A. **99**, 679 (2002).
[11] C. C. Gust, M. B. Elowitz, W. Hsing, and S. Leibler, Science **296**, 1466 (2002).
[12] L. Chen and K. Aihara, IEEE Trans. Circuits Syst., I: Fundam. Theory Appl. **49**, 602 (2002).
[13] L. Chen and K. Aihara, IEEE Trans. Circuits Syst., I: Fundam. Theory Appl. **49**, 1429 (2002).
[14] T. Kobayashi, L. Chen, and K. Aihara, in *Proceedings of 2002 IEEE Computer Society Bioinformatics Conference* (IEEE, New York, 2002), p. 151.
[15] T. Kobayashi, L. Chen, and K. Aihara, J. Theor. Biol. **221**, 379 (2003).
[16] S. Agha-Mohammadi and M. T. Lotze, J. Clin. Invest. **105**, 1177 (2000).
[17] S. Moser, M. Rimann, C. Fux, S. Schlatter, J. Bailey, and M. Fussenegger, J. Gene Med. **3**, 529 (2001).
[18] W. Weber and M. Fussenegger, J. Biotechnol. **98**, 161 (2002).
[19] R. Wang, T. Zhou, Z. Jing, and L. Chen, Syst. Biol. (to be published).
[20] M. B. Elowitz., A. J. Levine, E. D. Siggia, and P. S. Swain, Science **297**, 1183 (2002).
[21] E. M. Ozbudak, M. Thattai, I. Kurtser, A. D. Grossman, and A. V. Oudenaarden, Nat. Genet. **31**, 69 (2002).
[22] P. S. Swain, M. B. Elowitz, and E. D. Siggia, Proc. Natl. Acad. Sci. U.S.A. **99**, 12795 (2002).
[23] A. Goldbeter, Proc. Natl. Acad. Sci. U.S.A. **88**, 9107 (1991).
[24] J. J. Tyson and B. Novak, J. Theor. Biol. **210**, 249 (2001).
[25] B. Alberts, D. Bray, A. Johnson, J. Levis, M. Raff, K. Boberts, and P. Walter, *Essential Cell Biology* (Garland, New York, 1998).
[26] T. Matsuo, S. Yamaguchi, S. Mitsui, A. Emi, F. Shimoda, and H. Okamura, Science **302**, 255 (2003).
[27] L. Chen and K. Aihara, in Proceedings of ICSB (unpublished), p. 92.
[28] Y. Morishita and K. Aihara, J. Theor. Biol. **228**, 315 (2004).

[29] N. van Kampen, *Stochastic Processes in Physics and Chemistry* (North-Holland, Amsterdam, 1992).
[30] J. Pogliano, T. Q. Ho, Z. Zhong, and D. R. Hellinski, Proc. Natl. Acad. Sci. U.S.A. **98**, 4486 (2001).
[31] G. R. Plague, C. Dale, and N. A. Moran, Mol. Ecol. **12**, 1095 (2003).
[32] G. P. Jiang, K. S. Tang, and G. Chen, Chaos, Solitons Fractals **15**, 925 (2003).
[33] J. Guckenheimer and P. Holmes, *Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields* (Springer-Verlag, Berlin, 1983).
[34] V. Noireaux, R. Bar-Ziv, and A. Libchaber, Proc. Natl. Acad. Sci. U.S.A. **100**, 12672 (2003).
[35] T. Kondo and M. Ishiura, BioEssays **22**, 10 (2000).