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Yutaka HORI, Tae-Hyoung KIM and Shinji HARA

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DEPARTMENT OF MATHEMATICAL INFORMATICS
GRADUATE SCHOOL OF INFORMATION SCIENCE AND TECHNOLOGY
THE UNIVERSITY OF TOKYO
BUNKYO-KU, TOKYO 113-8656, JAPAN

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Graphical and Analytic Criteria for the Existence of Protein Level Oscillations in Cyclic Gene Regulatory Networks

Yutaka HORI*, Tae-Hyoung KIM† and Shinji HARA‡

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Abstract

This paper investigates the criteria for the existence of periodic oscillations of protein concentrations in a class of gene regulatory networks. There are two steps to derive such criteria: We first employ a Poincaré-Bendixson type theorem to restrict the class of solution trajectories, and then carry out a local stability analysis of linearized systems with a generalized frequency variable. Then, our main results, graphical and its equivalent analytic criteria which are easily applicable to the networks consisting of arbitrary number of genes with homogeneous dynamics, are derived. Their distinctive features are also verified through numerical simulations. Finally, we briefly discuss how to analyze the case where genes have heterogeneous dynamics, which makes the analysis problem considerably difficult since the number of free parameters increases.

1 INTRODUCTION

Recent study in molecular biology has revealed that protein level oscillations in a cell relates to the periodic phenomena in living organisms, such as the day and night cycle called the circadian rhythm. Although further study is still required to fully understand the phenomena, intensive efforts including theoretical as well as experimental approaches have been made to unveil the

*Department of Information Physics and Computing, Graduate School of Information Science and Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan. E-mail: Yutaka_Hori@ipc.i.u-tokyo.ac.jp

†School of Mechanical Engineering, Chung-Ang University, 221 Heukseok-dong, Dongjak-gu, Seoul 156-756, Korea. E-mail: kimth@cau.ac.kr

‡Department of Information Physics and Computing, Graduate School of Information Science and Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan. E-mail: Shinji_Hara@ipc.i.u-tokyo.ac.jp

mechanism of the oscillations. One of the remarkable approaches is synthetic biology, which attempts to artificially synthesize the genetic chemical reaction, and observe the underlying design principle of the living cells by the forward engineering approach. The Repressilator [1], the *small-scale* synthetic gene circuit consisting of three genes, was the pioneering work of a synthetic biological oscillator, and gave not only biological insights, but also the possibility of the engineering application of the protein level oscillator. In this line of researches, theoretical analyses based on mathematical models of complex biological systems are essential to clarify the relation between the system's parameters and the behavior of protein concentrations, and further to possibly predict other shadowy phenomena.

From the above backgrounds, it has been strongly required in this research field to develop unified simple methodologies for examining how such protein level oscillations occur in *large-scale* biological networks with arbitrary number of genes. This paper is concerned with an efficient way to develop such an analysis scheme from the control theoretical viewpoint.

The process by which gene information is converted for producing cell structures and cell functions is called gene expression, and is consisting of the two main process events, transcription and translation. During transcription some special blocks of the DNA, called genes, are copied into messenger RNA (mRNA), a molecule which serves as a template for the production of proteins. Then, a mRNA is decoded to make the corresponding protein during the translation process. Further, the transcription of a gene can be repressed or activated by regulatory proteins, called transcription factors. Such processes and interactions are referred to as the gene regulatory network, and are considered to control the various functions of living organisms. One of the earliest qualitative analyses of such gene regulatory networks is carried out by Goodwin [2], which introduced the dynamical model of the cyclic gene regulatory network where a transcription protein cyclically represses the transcription of a gene. Since the cyclic interaction is one of the fundamental patterns observed in the gene regulatory network as well as metabolic pathways [3] and cellular signaling pathways [4], the properties of the cyclic gene regulatory network were investigated in many later researches (see [5, 6, 7, 8] and references therein).

Regarding the protein level oscillations, Hastings *et al.* [9] presented the general condition for the existence of periodic oscillations in cyclic gene regulatory networks with only activations except one interaction, which is checked by the eigenvalue positions of the Jacobian matrix of the dynamics. Later, the above condition was further generalized by Mallet-Paret and Smith [10] to the class of cyclic gene regulatory networks with arbitrary combination of activations and repressions. In contrast to the Hopf bifurcation approaches [11], these conditions are obtained based on global analysis,

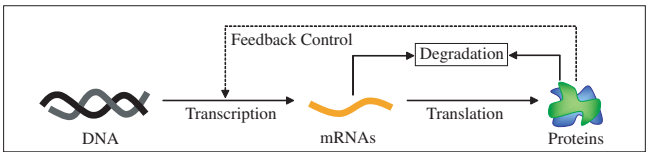


Figure 1: Mechanism of protein synthesis in gene regulatory networks

and thus the global properties of cyclic gene regulatory networks can be studied. Samad *et al.* [12] extended the dynamical model of the Repressilator to the arbitrary number of genes, and presented the analytic criteria of the existence of periodic oscillations based on the result in Hastings *et al* [9]. These criteria were obtained by the direct calculation of the eigenvalues of the Jacobian matrix. However, this approach generally has the potential drawback that the computation of the eigenvalue gets hard as the number of genes increases and the degree of the Jacobian becomes high. Therefore, it is expected to develop a novel analysis method which does not highly depend on the degree of the gene regulatory network system.

In this paper, we present graphical and analytic criteria for the existence of periodic oscillations in a class of the cyclic gene regulatory networks investigated in Samad *et al.* [12]. In order to study the dynamical behavior of the gene regulatory network system, we first introduce the dynamical model described by nonlinear differential equations. Then, based the result in Mallet-Paret and Smith [10], we specify the class of the omega-limit set of this system, and show that the omega-limit set, in fact, consists only of an equilibrium state or periodic oscillations. From this observation, we show that instability of the equilibrium state is the key property for oscillations in cyclic gene regulatory networks. Thus, we next analyze the local stability of an equilibrium state. For this purpose, we first consider a class of the gene regulatory networks where every gene has the homogeneous dynamics, and then present that its linearized system belongs to a class of large-scale systems with a generalized frequency variable [13, 14]. Finally, the graphical criterion is presented based on the results in Hara *et al.* [13, 14], and then its equivalent analytic criterion is derived from the geometric consideration of the graphical one. These criteria have the feature that the analysis does not highly depend on the number of genes, and thus can be easily applied to the gene regulatory network consisting of large number of genes. Finally, we briefly discuss the case where genes have heterogeneous dynamics, which makes the analysis problem considerably difficult since the number of free parameters increases.

In this paper, we define $\mathbb{R}_+ := \{x \in \mathbb{R} \mid x \geq 0\}$ and $\mathbb{C}_+ := \{x \in \mathbb{C} \mid \mathrm{Re}[x] > 0\}$.

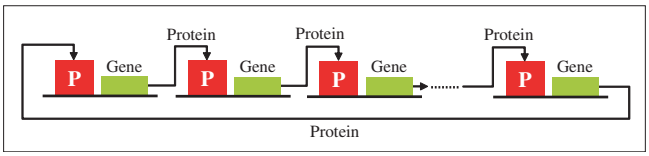


Figure 2: Dynamic gene regulatory network system with cyclic connections

2 Modeling of Gene regulatory networks and problem description

The well-known central dogma of molecular biology is that protein is synthesized following the two steps called transcription and translation: genes on a DNA are first transcribed into mRNAs, and then a mRNA is translated into one or multiple copies of corresponding proteins (see Fig. 1). Further, some proteins, called transcription factors, are known to activate or repress the transcription of other genes. Then, such chemical interactions between transcription factors and genes can be described by gene regulatory networks.

In this paper, we consider the gene regulatory network where a transcription factor of one gene activates or represses the transcription of another gene in a cyclic way as depicted in Fig. 1. Note that this cyclic feedback structure is one of the substantial chemical pathways in living organisms, and is also observed in metabolic pathways, tissue growth regulations, cellular signaling pathways and neuron models (see [15] and references therein). Then, the dynamics of the above cyclic gene regulatory networks is modeled as, for $i = 1, 2, \cdots, N$,

$$\begin{aligned} \dot{r}_i(t) &= -a_i r_i(t) + \beta_i f_i(p_{i-1}(t)), \\ \dot{p}_i(t) &= c_i r_i(t) - b_i p_i(t), \end{aligned} \tag{1}$$

where $r_i \in \mathbb{R}_+$ and $p_i \in \mathbb{R}_+$ denote the concentrations of the i-th mRNA and its corresponding protein synthesized in the i-th gene, respectively. Let $p_0(t) := p_N(t)$ and $r_0(t) := r_N(t)$ for the sake of notational simplification. Positive constants a_i, b_i, c_i and β_i represent the followings: a_i and b_i denote the degradation rates for the i-th mRNA and protein, respectively. c_i and β_i denote the translation and transcription rates, respectively. A monotonic function $f_i(\cdot) : \mathbb{R}_+ \to \mathbb{R}_+$ represents either activation or repression of the transcription. It is defined for repression as $f_i(0) = 1$ and $f_i(\infty) = 0$ (monotone decreasing), whereas for activation $f_i(0) = 0$ and $f_i(\infty) = 1$ (monotone increasing). As one of the candidates for $f_i(\cdot)$, the following Hill functions are often introduced in biochemical characterization:

$$f_i(p_{i-1}) = \frac{1}{1 + p_{i-1}^{\nu}}, \quad f_i(p_{i-1}) = \frac{p_{i-1}^{\nu}}{1 + p_{i-1}^{\nu}}, \tag{2}$$

where ν is the Hill coefficient and determines the nonlinearity of the system [16]. Let the following assumption be satisfied throughout this paper:

Assumption 1. *For given $f_i(\cdot)$ ($i = 1, 2, \cdots, N$), it holds that*

$$\delta := \left(\frac{df_1}{dp_N}\right) \cdot \left(\frac{df_2}{dp_1}\right) \cdots \left(\frac{df_N}{dp_{N-1}}\right) < 0. \tag{3}$$

It means that a given gene regulatory network has an odd number of repressive interactions between genes. Note that this assumption is considered to be acceptable, because *almost all* solutions of (1) are observed to asymptotically converge to one of the equilibria in the case of $\delta > 0$ (refer to [7]).

Gene regulatory network systems are generally large-scale and further contain nonlinearity such as $f_i(\cdot)$. It is thus required to develop considerably simple methods and tools which can explicitly analyze the complex dynamical behavior. Therefore, the research objective of this paper is summarized as follows.

Problem: Derive a graphical criterion for the existence of protein level's periodic oscillations in a class of large-scale cyclic gene regulatory networks, where all genes are assumed to have identical dynamics. Then, derive an equivalent analytic criterion based on the above results.

Note that the assumption that all genes have identical dynamics is plausible, because genes on a DNA consist of nearly the same bases.[1]

3 Characterization of omega-limit set of cyclic gene regulatory network systems

In this section, we examine the omega-limit set of cyclic gene regulatory networks modeled by nonlinear differential equations in (1). Specifically, we will show that the omega-limit set of (1), in fact, consists of either an equilibrium state or limit cycles. Consider the following key result given by Mallet-Paret and Smith [10] (see Theorem 4.1 in [10]):

Proposition 1. *For the system (1), the omega-limit set consists of either (i) an equilibrium state $\boldsymbol{z}^* := [r_1^*, p_1^*, r_2^*, p_2^*, \cdots, r_N^*, p_N^*] \in \mathbb{R}^{2N}$, (ii) a non-constant periodic orbit, or (iii) $\boldsymbol{z}^*$ together with a collection of orbits homoclinic to $\boldsymbol{z}^*$, if all the following three conditions (a), (b) and (c) hold:*

[1] In some cases, the components(genes) in gene regulatory networks may be somewhat heterogeneous, which makes it far more difficult to characterize and analyze its dynamical behavior. The existence of protein level's periodic oscillations in this case will be discussed in Section 6.

(a) $\mathbb{R}^{2N}_+$ *is a positively invariant set,*

(b) Positive semiorbit $\{\boldsymbol{z}(t) \mid t \geq 0 \text{ and } t \in \text{dom } \boldsymbol{z}(\cdot)\} \subset \mathbb{R}^{2N}_+$ *is bounded,*

(c) There is a unique equilibrium state $\boldsymbol{z}^*$.

Furthermore, if

(d) $\det(-J) > 0$,

also holds, then either (i) or (ii) occurs where J *is the Jacobian matrix of the system.*

This proposition restricts a class of omega-limit set of the system, and rules out the chaotic behavior of the solution as in the Poincaré-Bendixson theorem for two dimensional systems. In the following, we show that (1) satisfies the above conditions (a), (b), (c) and (d).

Regarding (a) and (b), Samad *et al.* [12] presented the following result (see Lemma 3 in [12]).

Lemma 1. *For the system (1),* $\mathbb{R}^{2N}_+$ *is a positively invariant set. In particular, all orbits starting from* $\boldsymbol{z}(0) \in \mathbb{R}^{2N}_+$ *converge to the set* $\mathcal{S}$*, where*

$$\mathcal{S} := \Big\{ (r_1, p_1, r_2, p_2, \cdots, r_N, p_N) \in \mathbb{R}^{2N}_+ \Big| \\ 0 \leq r_i \leq \frac{\beta_i}{a_i},\ 0 \leq p_i \leq \frac{c_i \beta_i}{a_i b_i},\ i = 1, 2, \cdots, N \Big\}.$$

This lemma implies that all trajectories $\boldsymbol{z}(\cdot)$ starting from $\mathbb{R}^{2N}_+$ are bounded, and thus the conditions (a) and (b) hold for the system (1).

Next, we consider the number of equilibria of the system (1). Since $\dot{p}_i = \dot{r}_i = 0$ for $i = 1, 2, \cdots, N$ at an equilibrium state, we have

$$p_i^* = (R_i^2 f_i) \circ (R_{i-1}^2 f_{i-1}) \cdots\cdots \circ (R_1^2 f_1) \circ (R_N^2 f_N) \cdots (R_{i+1}^2 f_{i+1})(p_i^*), \qquad (4)$$

where

$$R_i := \frac{\sqrt{c_i \beta_i}}{\sqrt{a_i b_i}}, \qquad (5)$$

and the notation $\circ$ denotes the composition of functions. Note that R_i is the ratio between the geometric means of degradation and production rates. Then, from the monotonicity of the both sides of (4), we obtain the following lemma.

Lemma 2. *The system in (1) has a unique positive equilibrium state.*

Note that the equation (4) is generally highly complicated, but the solution can be efficiently obtained by using the bisectional search algorithm and the

monotonicity property of (4). In particular, if $f_i(p_{i-1}) = 1/(1+p_{i-1}^{\nu})$, and $a_i = a$, $b_i = b$, $c_i = c$, $\beta_i = \beta$ for $\forall i$ (i.e., $R_1 = \cdots R_N = R$) hold, then we have a much simpler equation

$$p_i^* = Rf_i(p_i^*),\ i = 1, 2, \cdots, N, \tag{6}$$

which implies $p_1^* = \cdots = p_N^*$, and $r_1^* = \cdots = r_N^*$.

As for the condition (d), we can see the following result (see Remark 4.1 in [10]):

Lemma 3. *Let J denote the Jacobian of (1). Then,* $\det(-J) > 0$ *holds.*

We can readily verify this lemma, since the form of

$$J = \begin{bmatrix} -a_1 & 0 & \cdots & \cdots & \beta_1 f_1'(p_N^*) \\ c_1 & -b_1 & \ddots & \ddots & 0 \\ 0 & \beta_2 f_2'(p_1^*) & -a_2 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & \cdots & c_N & -b_N \end{bmatrix},$$

yields

$$\det(-J) = \prod_{i=1}^{N} a_i b_i - \prod_{i=1}^{N} c_i \beta_i f_i'(p_{i-1}^*), \tag{7}$$

and Assumption 1 implies that the second term of the right-hand side in (7) becomes negative.

We can conclude from the above observations that the conditions (a), (b), (c) and (d) in Proposition 1 hold for the system (1). Thus, it follows that the protein concentrations p_i $(i = 1, 2, \cdots, N)$ of the considered cyclic gene regulatory network system either (i) converge to a unique equilibrium state, or (ii) oscillate periodically.

4 Main results

In this section, we derive both graphical and analytic criteria for the existence of protein level's periodic oscillations in large-scale cyclic gene regulatory networks.

4.1 Graphical criterion for the existence of oscillations

As mentioned in Section 3, protein concentrations of the cyclic gene regulatory network either (i) converge to an equilibrium state, or (ii) oscillate periodically. Thus, if such a unique equilibrium state is locally unstable, there

exists a set of initial values so that protein concentrations do not converge to the equilibrium level and eventually enter into a non-constant periodic orbit. Note that conventional analysis methods may be available to check the local stability of the equilibrium state. These approaches, however, have a potential drawback such that the computational burden becomes excessive as the number of genes gets large and the degree of the system increases. Therefore, it is desirable to develop a reasonable analysis method whose computation burden does not highly depend on the number of genes, which is one of our main research objectives.

In order to analyze the local stability of (1), we now consider its linearized model around the equilibrium state $\boldsymbol{z}^*$:

$$\begin{bmatrix} \dot{r}_i \\ \dot{p}_i \end{bmatrix} = \begin{bmatrix} -a_i & 0 \\ c_i & -b_i \end{bmatrix} \begin{bmatrix} r_i \\ p_i \end{bmatrix} + \begin{bmatrix} \beta_i \\ 0 \end{bmatrix} u_i, \ u_i := \zeta_i p_{i-1}, \tag{8}$$

where

$$\zeta_i := f_i'(p_{i-1}^*). \tag{9}$$

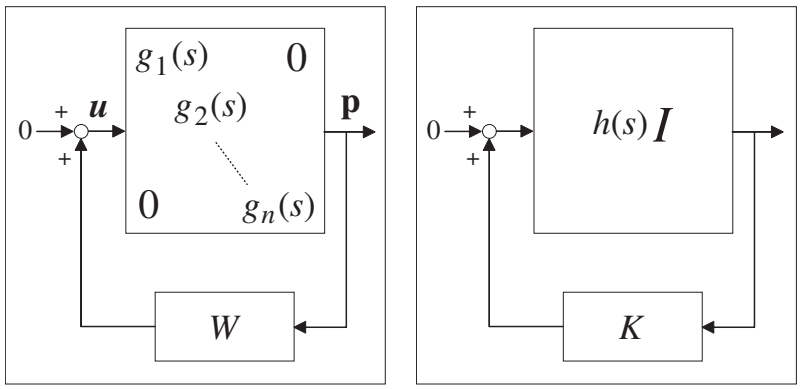
Note that Assumption 1 is equivalent to $\prod_{i=1}^{N} \zeta_i < 0$. Hence, mRNA and protein concentrations of the i-th gene, r_i and p_i in (8), can be interpreted from the control theoretic viewpoint as the internal states of the system. Also, we can see from the definition of u_i that the $(i-1)$-th protein concentration has an influence (activation or repression) on the transcription rate of the i-th mRNA production, which results from the cyclic structure of gene regulatory networks in Fig. 1. Thus, the transfer function for the i-th gene, $g_i(s)$, from the input u_i to the protein concentration p_i is obtained as follows:

$$g_i(s) := \frac{c_i \beta_i}{(s+a_i)(s+b_i)} = \frac{R_i^2}{(T_{a_i} s + 1)(T_{b_i} s + 1)}, \tag{10}$$

where $T_{a_i} = 1/a_i$ and $T_{b_i} = 1/b_i$ for $i = 1, 2, \cdots, N$. Consequently, the overall dynamics of the cyclic gene regulatory network is expressed as shown in Fig. 3(a), where $\boldsymbol{u} = W\boldsymbol{p}$, $\boldsymbol{u} := [u_1, u_2, \cdots, u_N]^T \in \mathbb{R}^N$ with

$$W := \begin{bmatrix} 0 & 0 & 0 & \cdots & \zeta_1 \\ \zeta_2 & 0 & 0 & \cdots & 0 \\ 0 & \zeta_3 & 0 & \cdots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & \zeta_N & 0 \end{bmatrix}. \tag{11}$$

Since the feedback gain matrix W has a special structure, the gains R_i^2 in $g_i(s)$ can be merged into the corresponding feedback gains in W. Moreover, it can be assumed that $a_1 = \cdots = a_N (=: a)$ and $b_1 = \cdots = b_N (=: b)$ because



(a) The overall gene regulatory network system $\mathcal{G}(s)$

(b) The modified gene regulatory network system $\mathcal{H}(s)$, which is equivalent to the one in Fig. 3(a).

Figure 3: Block diagram of the linearized gene regulatory network systems

the DNA consist of nearly the same bases as mentioned in Section 2. Then, the feedback system shown in Fig. 3(a) can be transformed into a feedback system depicted in Fig. 3(b), where

$$h(s) := \frac{1}{(T_a s + 1)(T_b s + 1)} \tag{12}$$

with $T_a := 1/a$, $T_b = 1/b$, and

$$K := \begin{bmatrix} 0 & 0 & 0 & \cdots & \zeta_1 R_1^2 \\ \zeta_2 R_2^2 & 0 & 0 & \cdots & 0 \\ 0 & \zeta_3 R_3^2 & 0 & \cdots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & \zeta_N R_N^2 & 0 \end{bmatrix}. \tag{13}$$

Therefore, the overall transfer function $\mathcal{H}(s)$ is obtained as

$$\mathcal{H}(s) := (\phi(s)I - K)^{-1}, \quad \phi(s) := 1/h(s). \tag{14}$$

Note that $\mathcal{H}(s)$ belongs to a class of large-scale linear systems with a generalized frequency variable $\phi(s)$, for which the useful stability analysis method is proposed in [13] and [14].

Based on the above observations and the stability analysis scheme in [13] and [14], a graphical criterion for the existence of protein level's periodic oscillations in a class of large-scale cyclic homogeneous gene regulatory network systems is obtained as follows:

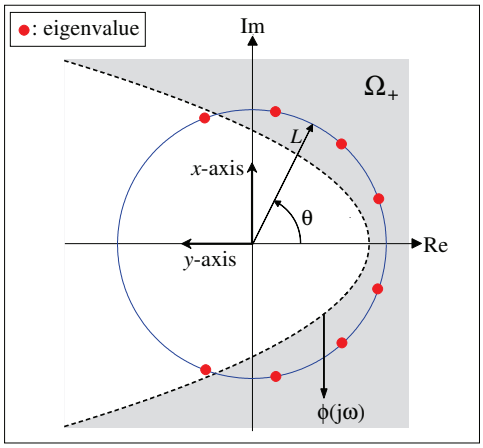


Figure 4: The domain Ω_+ and the eigenvalue distribution of K.

Theorem 1. *Consider the cyclic gene regulatory network with gene dynamics (1) where $a_i = a$, $b_i = b$ $(i = 1, 2, \cdots, N)$, and its linearized system $\mathcal{H}(s)$ in (14). Assume that the condition in (3) holds; i.e., $\Pi_{i=1}^{N} \zeta_i < 0$. Then, if at least one of the eigenvalues of K in (13) exists within the domain Ω_+ defined as*

$$\Omega_+ := \phi(\mathbb{C}_+) = \{\lambda \in \mathbb{C} \mid \exists s \in \mathbb{C}_+ \text{ s.t. } \phi(s) = \lambda\},$$

there exist the periodic oscillations of protein concentrations p_i $(i = 1, 2, \cdots, N)$.

This graphical criterion means that the stability of $\mathcal{H}(s)$ can be easily checked by the eigenvalue distribution of K which expresses the interaction between genes, and the domain Ω_+ which is determined by the identical gene dynamics $h(s)$. Specifically, in this case, the eigenvalues of K are simply computed as, for $k = 1, 2, \cdots, N$,

$$\lambda_k = L e^{j(2k-1)\pi/N}, \quad L := \left| \prod_{\ell=1}^{N} R_\ell^2 \zeta_\ell \right|^{\frac{1}{N}}. \tag{15}$$

It implies that all eigenvalues of K are located on a circumference with a center at the origin and a radius of L. On the other hand, we can see from (12) that the border of Ω_+ draws parabolic curve (see Fig. 4). Therefore, the existence of protein level's periodic oscillations can be easily confirmed by just using the above mentioned two elementary shapes. In the sequel, we see that the graphical criterion in Theorem 1 can be equivalently rewritten in an analytic form.

4.2 Analytic criterion for the existence of oscillations

In this subsection, we first derive an analytic expression describing the boundary of domain Ω_+, and then derive an analytic criterion for checking the existence of protein level's periodic oscillations in (1).

Let the x-y coordinate be defined as illustrated in Fig. 4, Then, the domain Ω_+ can be characterized in terms of $x(\omega) := \mathrm{Im}[\phi(j\omega)]$ and $y(\omega) := -\mathrm{Re}[\phi(j\omega)]$ as

$$\Omega_+ := \left\{ -y + jx \in \mathbb{C} \mid y < \frac{1}{4}Q^2x^2 - 1 \right\}, \tag{16}$$

where

$$Q := \frac{\sqrt{T_aT_b}}{(T_a+T_b)/2} \left(= \frac{\sqrt{ab}}{(a+b)/2} \right). \tag{17}$$

Note that Q is the ratio between the arithmetic and geometric means of mRNA and protein time constants, T_a and T_b.

Then, based on the above observation and the specific eigenvalue distribution of K in (15), we have the following analytic criterion for the existence of periodic oscillations:

Theorem 2. *Consider the cyclic gene regulatory network with gene dynamics (1) where $a_i = a$, $b_i = b$, $(i = 1, 2, \cdots, N)$, and its linearized system $\mathcal{H}(s)$ in (14). Assume that the condition in (3) holds; i.e., $\Pi_{i=1}^N \zeta_i < 0$. Then, there exist the periodic oscillations of protein concentrations p_i $(i = 1, 2, \cdots, N)$, if*

$$L > \frac{2\left(-\cos(\frac{\pi}{N}) + \sqrt{\cos^2(\frac{\pi}{N}) + Q^2\sin^2(\frac{\pi}{N})}\right)}{Q^2\sin^2(\frac{\pi}{N})}. \tag{18}$$

In (18), the left-hand side stands for the radius of the circle where all eigenvalues are located. On the other hand, the right-hand side is the distance from the origin to the boundary $\phi(j\omega)$ which goes through the eigenvalue λ_1 (i.e., $\theta = \pi/N$). Note that this distance achieves a minimum at $\theta = 0$, and monotonically increases as θ increases. The above fact means that λ_1 and λ_N in (15) always get into the domain Ω_+ first as the radius of the circle increases (see Fig. 5). Therefore, the above condition (18) is equivalent to the graphical one in the Theorem 1.

Note that if we make the additional assumption such that $T_a = T_b$, $c_1 = \cdots = c_N$, $\beta_1 = \cdots = \beta_N$ and $f_i(p_{i-1}) = 1/(1 + p_{i-1}^\nu)$ $(i = 1, \cdots, N)$,

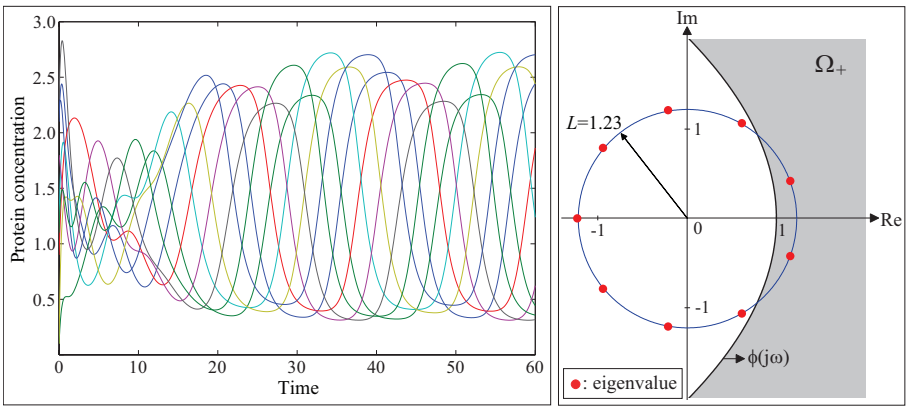


Figure 5: (Right) The domain Ω_+ and the eigenvalues of K: Two eigenvalues belong to Ω_+. (Left) Time plot of oscillatory protein concentrations

the analytic condition which explicitly considers the relation between the parameters and the equilibrium point $\boldsymbol{z}^*$ is obtained (refer to [17]). In other words, it is analytically written only in terms of the given parameters (N, ν, R).

Finally, we briefly remark on the relation between our results and the conventional ones. The analysis by Samad *et al.* [12] was performed based on the direct computation of the eigenvalues of the Jacobian matrix, and then analytic criteria for $N = 2, 3$ were presented. We can easily see that their results coincide with the ones in Theorem 2 with $N = 2, 3$.

5 Numerical example

Consider the cyclic gene regulatory network composed of $N = 9$ genes with dynamics in (1). Assume that $a_i = 1.4$, $b_i = 2.0$ for $i = 1, 2, \cdots, 9$, $c_1 = c_3 = c_5 = c_6 = c_8 = 3.3$, $c_2 = c_7 = 2.9$, $c_4 = c_9 = 3.8$ and $\beta_1 = \beta_5 = \beta_6 = 2.4$, $\beta_2 = \beta_7 = \beta_8 = 2.5$, $\beta_3 = \beta_4 = \beta_9 = 2.3$. Let the hill function be defined as $f_i(p_{i-1}) = 1/(1 + p_{i-1}^{\nu})$ with $\nu = 2.1$ for $i = 1, 2, \cdots, 9$, which represents that the $(i-1)$-th protein represses the transcription of the i-th gene. Then, the equilibrium state is computed from (4) as $\boldsymbol{z}^* = [0.731, 1.21, 0.720, 1.04, 0.785, 1.29, 0.604, 1.15, 0.734, 1.21, 0.687, 1.13, 0.776, 1.13, 0.782, 1.29, 0.606, 1.15]^T$. Thus, simple calculation yields $L = 1.23$.

We first verify the effectiveness of the result in Theorem 1 (graphical criterion). In this case, the domain Ω_+ is determined by using $h(s)$ in (12). On the other hand, the distribution of eigenvalues of K can be easily found: i.e., these are located at equiangularly spaced points on the circumference with radius of $L = 1.23$ as shown in (15) (See Fig. 5(Right)). Since two eigenvalues belong to the domain Ω_+ in Fig. 5(Right), we can readily con-

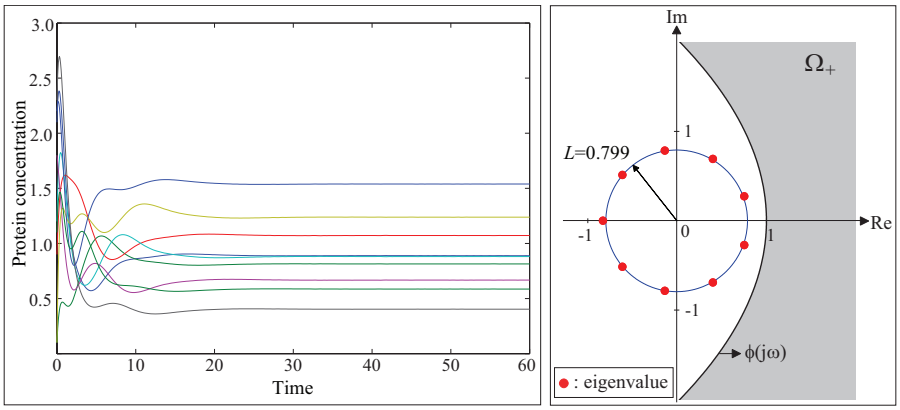


Figure 6: (Right) The domain Ω_+ and the eigenvalues of K: All eigenvalues are located outside Ω_+. (Left) Plot showing the convergence of protein concentrations to a unique equilibrium state

clude based on Theorem 1 that the gene regulatory network has the periodic oscillations of protein concentrations p_i $(i = 1, 2, \cdots, 9)$, which is confirmed by Fig. 5(Left), where the initial values of r_i and p_i are set as follows: $r_1(0) = 2.0$, $r_2(0) = 0.6$, $r_3(0) = 1.2$, $r_4(0) = 1.5$, $r_5(0) = 0.9$, $r_6(0) = 1.6$, $r_7(0) = 2.6$, $r_8(0) = 1.7$, $r_9(0) = 1.2$, $p_1(0) = 2.1$, $p_2(0) = 0.1$, $p_3(0) = 0.9$, $p_4(0) = 1.2$, $p_5(0) = 1.8$, $p_6(0) = 0.1$, $p_7(0) = 2.3$, $p_8(0) = 2.2$, $p_9(0) = 1.1$.

Next, we show that the same result can be found by using Theorem 2 (analytic criterion). In (18), $L = 1.23$ as mentioned above, and the right-hand side is given by

$$\frac{2\left(-\cos(\frac{\pi}{9}) + \sqrt{\cos^2(\frac{\pi}{9}) + Q^2\sin^2(\frac{\pi}{9})}\right)}{Q^2\sin^2(\frac{\pi}{9})} = 1.03 \tag{19}$$

Therefore, the condition (18) holds, which implies the existence of periodic oscillations of protein concentrations.

On the other hand, if β_i is set as $\beta_1 = \beta_5 = \beta_7 = 1.0, \beta_2 = \beta_4 = 1.4, \beta_3 = \beta_6 = \beta_8 = \beta_9 = 1.5$, a unique equilibrium state becomes $\boldsymbol{z}^* = [0.539, 0.889, 0.561, 0.814, 0.650, 1.07, 0.464, 0.881, 0.404, 0.667, 0.750, 1.24, 0.278, 0.404, 0.933, 1.54, 0.309, 0.586]^T$, and hence the eigenvalue distribution of K becomes as shown in Fig. 6(Right). Since all the eigenvalues are located outside the domain Ω_+ in this case, the existence of periodic oscillations of p_i is not guaranteed in the system (See Fig. 6(Left)). The above fact can also be confirmed by using the result in Theorem 2; i.e., the condition in (18) is not guaranteed, since $L = 0.799$ and the right-hand side of (18) is 1.03.

6 Discussion: Oscillations in gene regulatory networks with heterogeneous gene dynamics

In the previous section, we studied the existence of periodic oscillations of protein concentrations in homogeneous gene regulatory network systems (i.e., $a_i = a$ and $b_i = b$ for $i = 1, 2, \cdots, N$). However, in some cases, the genes in this large and complex system may be somewhat heterogeneous as mentioned in Section 2. Thus, a theoretical criterion for the existence of periodic oscillations in heterogeneous case is briefly discussed.

We hereafter focus on the case where each parameter's value a_i, b_i, c_i and β_i is not necessarily identical to each other, respectively, and only the upper and lower bounds of each parameter are given by $\underline{a} \leq a_i \leq \overline{a}$, $\underline{b} \leq b_i \leq \overline{b}$, $\underline{c} \leq c_i \leq \overline{c}$ and $\underline{\beta} \leq \beta_i \leq \overline{\beta}$ for $i = 1, 2, \cdots, N$. On the other hand, a thorough study of perturbation of ζ_i in W is demanding, because ζ_i is determined by the equilibrium state $\boldsymbol{z}^*$, which takes the effect of the changes of a_i, b_i, c_i and β_i. Hence, we here assume that the perturbation range of $\zeta_i (i = 1, 2, \cdots, N)$ is given in advance by $\underline{\zeta} \leq |\zeta_i| \leq \overline{\zeta}$, which covers not only the effect of the change of the equilibrium state, but also the deviation of the Hill functions $f_i(\cdot) (i = 1, 2, \cdots, N)$. It follows from the above assumption that we can regard a_i, b_i, c_i, β_i and ζ_i $(i = 1, 2, \cdots, N)$ as the independent parameters.

Then, it follows from the results in Section 4 that if a unique equilibrium state of cyclic gene regulatory networks with heterogeneous parameters a_i, b_i, c_i, β_i and ζ_i becomes unstable, there exist periodic oscillations of protein concentrations. Therefore, we investigate the worst case parameter values of $(a_i, b_i, c_i, \beta_i, \zeta_i)$ which guarantee the existence of periodic oscillations as shown in the following.

As shown in (10), the transfer function of the i-th gene's dynamics is obtained as $g_i(s) = c_i\beta_i/(s + a_i)(s + b_i)$. Then, the overall system of a class of heterogeneous gene regulatory networks, $\mathcal{G}(s) := (\hat{\Phi}(s) - W)^{-1}$, can be represented as depicted in Fig. 3(a), where $\hat{\Phi}(s) := \mathrm{diag}(1/g_1(s), 1/g_2(s), \cdots, 1/g_N(s))$ and W is as defined in (11). Note that the characteristic polynomial of $\mathcal{G}(s)$ is written as

$$\frac{1}{c_1\beta_1}(s + a_1)\gamma(s) + v = 0, \tag{20}$$

where

$$\gamma(s) := (s + b_1)\prod_{i=2}^{N}\frac{(s + a_i)(s + b_i)}{c_i\beta_i} \tag{21}$$

and $v := |\prod_{i=1}^{N}\zeta_i|$.

Then, we consider the worst case values of $(a_i, b_i, c_i, \beta_i, \zeta_i)$ for instability of $\mathcal{G}(s)$. First, it is obvious that for any given (a_i, b_i, c_i, β_i), the system tends to be unstable as v gets large, because v can be regarded as the feedback gain of $\mathcal{G}(s)$. Thus, it follows that $\underline{v} := \prod_{i=1}^{N} \underline{\zeta}_i$ gives the worst case gain for instability of $\mathcal{G}(s)$.

Next, we investigate the other parameters (a_i, b_i, c_i, β_i), and obtain the worst case values for instability of $\mathcal{G}(s)$. It follows from the gain and phase conditions of (20) that for given (a_i, b_i, c_i, β_i), the critical gain v^* for instability can be expressed as

$$v^* = \frac{1}{c_1\beta_1}\sqrt{a_1^2 + \omega^2}|\gamma(j\omega)| \tag{22}$$

$$\text{such that } \angle(a_1 + j\omega) = \pi - \angle\gamma(j\omega). \tag{23}$$

Then, the following lemma gives an important relationship between the parameters (a_i, b_i, c_i, β_i) and the critical gain v^*.

Lemma 4. *Consider the linearized gene regulatory network system given by $\mathcal{G}(s)$. Then, the critical gain v^* for instability of $\mathcal{G}(s)$ monotonically increases with respect to a_i and b_i $(i = 1, 2, \cdots, N)$, and monotonically decreases with respect to c_i and β_i $(i = 1, 2, \cdots, N)$.*

Proof: We first assume that the parameters a_i $(i = 2, 3, \cdots, N)$ and b_i, c_i, β_i $(i = 1, 2, \cdots, N)$ are fixed, and concentrate on the perturbation of a_1.

Let $a_\rho (= a_1)$ and $\omega_\rho (= \omega)$ satisfy both (22) and (23), and v_ρ^* be the critical gain for instability of $\mathcal{G}(s)$ with (a_ρ, ω_ρ). In addition, let a_ν be a certain positive constant satisfying $a_\nu > a_\rho$. Then, it is obvious that

$$\angle(a_\nu + j\omega_\rho) < \angle(a_\rho + j\omega_\rho) = \pi - \gamma(j\omega_\rho). \tag{24}$$

Since $\angle\gamma(j\omega)$ is a monotonically increasing function with respect to ω, it follows from (24) that there exists $\omega_\nu (> \omega_\rho)$ such that $\angle(a_\nu + j\omega_\nu) = \pi - \gamma(j\omega_\nu)$. Then, the critical gain v_ν^* for instability of $\mathcal{G}(s)$ with (a_ν, ω_ν) is obtained as

$$v_\nu^* = \frac{1}{c_1\beta_1}\sqrt{a_\nu^2 + \omega_\nu^2}|\gamma(j\omega_\nu)|. \tag{25}$$

This implies $v_\nu^* > v_\rho^*$ because $a_\nu > a_\rho$ and $\omega_\nu > \omega_\rho$. Thus, the critical gain v^* monotonically increases with respect to a_1. For $a_2, a_3, \cdots, a_N$ and $b_1, b_2, \cdots, b_N$, the above conclusion is proved in a similar way.

On the other hand, since the phase condition (23) is not affected by the change of c_i and β_i $(i = 1, 2, \cdots, N)$, it immediately follows from (22) that the critical gain monotonically decreases with respect to c_i and β_i. □

The above lemma implies that the critical gain v^* achieves the maximum when $a_i = \overline{a}$, $b_i = \overline{b}$, $c_i = \underline{c}$ and $\beta_i = \underline{\beta}$ $(i = 1, 2, \cdots, N)$. In other words,

$\mathcal{G}(s)$ is most likely to be stable when $a_i = \overline{a}$, $b_i = \overline{b}$, $c_i = \underline{c}$ and $\beta_i = \underline{\beta}$ $(i = 1, 2, \cdots, N)$, and thus, this parameter set gives the worst case for instability of $\mathcal{G}(s)$. Therefore, the following criterion for the existence of periodic oscillations in cyclic gene regulatory networks with heterogeneous genes is obtained.

Proposition 2. *Consider the cyclic gene regulatory network with gene dynamics (1) and its linearized system $\mathcal{G}(s)$. Assume that the condition in (3) holds, i.e., $\Pi_{i=1}^{N}\zeta_i < 0$, and $\underline{a} \le a_i \le \overline{a}$, $\underline{b} \le b_i \le \overline{b}$, $\underline{c} \le c_i \le \overline{c}$, $\underline{\beta} \le \beta_i \le \overline{\beta}$ and $\underline{\zeta} \le |\zeta_i| \le \overline{\zeta}$ $(i = 1, 2, \cdots, N)$ are satisfied for given $\underline{a}$, $\overline{a}$, $\underline{b}$, $\overline{b}$, $\underline{c}$, $\overline{c}$, $\underline{\beta}$, $\overline{\beta}$, $\underline{\zeta}$ and $\overline{\zeta}$. Then, there exist the periodic oscillations of protein concentrations p_i $(i = 1, 2, \cdots, N)$ if the system $\mathcal{G}(s)$ with $a_i = \overline{a}, b_i = \overline{b}, c_i = \underline{c}, \beta_i = \underline{\beta}$ and $|\zeta_i| = \underline{\zeta}$ is unstable.*

The above proposition gives a sufficient condition for the existence of periodic oscillations for cyclic gene regulatory networks with heterogeneous gene dynamics. From the above proposition, we can see that the existence of periodic oscillations in *heterogeneous* cyclic gene regulatory networks can be confirmed just by checking the local stability of the *homogeneous* cyclic gene regulatory networks. Hence, we can introduce the simple graphical and analytic criteria given in Section 4. Specifically, if the condition in Theorem 1 or 2 holds for $a_i = \overline{a}, b_i = \overline{b}, c_i = \underline{c}, \beta_i = \underline{\beta}$ and $|\zeta_i| = \underline{\zeta}$ $(i = 1, 2, \cdots, N)$, then there exist periodic oscillations of protein concentrations p_i for any a_i, b_i, c_i and β_i satisfying $\underline{a} \le a_i \le \overline{a}$, $\underline{b} \le b_i \le \overline{b}$, $\underline{c} \le c_i \le \overline{c}$ and $\underline{\beta} \le \beta_i \le \overline{\beta}$. Nevertheless, in order to completely understand the periodic oscillations of protein concentrations in the heterogeneous cyclic gene regulatory networks, thorough study of the relation between the system parameters and the equilibrium point, which determines ζ_i, may be indispensable, and hence it is one of our future research works.

7 Conclusion

In this paper, we have investigated the existence condition of periodic oscillations for large-scale cyclic gene regulatory networks whose dynamics is modeled by the nonlinear differential equations. To this end, we first restricted the class of the omega-limit set by employing the Poincaré-Bendixson type theorem [10], and then presented that there exists periodic oscillations in the system if a unique equilibrium state is locally unstable. In general, as the number of genes increases, the stability analysis gets difficult, because the size of the Jacobian matrix grows in proportion to the number of genes. In order to overcome this problem, we introduced the stability analysis method for large-scale linear systems with a generalized frequency variable [13, 14], and presented the graphical and its equivalent analytic criteria for the existence of protein level's periodic oscillations. The developed criteria have a

remarkable feature that they are easily applicable to cyclic gene regulatory networks consisting of any number of genes. Finally, we briefly discussed the case where the dynamics of each gene is heterogeneous, and derived a criterion for the existence of periodic oscillations of protein concentrations, though it is somewhat conservative. Thus, comprehensive analysis method which can handle the heterogeneous parameter case will be studied in future work.

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