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Oscillations in Cyclic Gene Regulatory Networks with Time Delay: Analytic Existence Conditions with Biological Insight

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Abstract

In this paper, we present analytic conditions for the existence of oscillations in gene regulatory networks with cyclic structure. In particular, inherent time delays in transcription, translation and translocation process are explicitly treated, and the effects of such time delays are revealed. We first show that local instability of a unique equilibrium state results in oscillations of protein concentrations based on the Poincaré-Bendixson theorem for cyclic time delay systems. Then, an analysis scheme of local instability for large-scale time delay systems is introduced. Using this scheme, we derive graphical and analytic conditions for the existence of oscillations. The developed analytic conditions are represented only in terms of biochemical parameters, thus they are suitable for obtaining biological insights. The results are applied to existing genetic networks, and biological insights are demonstrated with illustrative numerical simulations.

1 Introduction

One of the main objectives of systems biology is to develop unified analysis schemes that can systematically examine the dynamical behaviors of large-scale gene regulatory networks. During the last decade, many theoretical works have been devoted to investigate the oscillatory as well as convergent behaviors of protein concentrations in living cells (see [12] and references therein).

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One of the pioneering theoretical analyses of gene regulatory networks was presented by Goodwin [7], where the dynamical model of cyclically interconnected gene's products was introduced. Later, the cyclic feedback structure was found in metabolic and cellular signaling pathways as well [13, 19], and it is recently considered that cyclic structure plays a key role to produce the various dynamical behaviors of protein levels (see Hori *et al.* [11] and references therein). In fact, the artificially constructed biological oscillator, named Repressilator [5], was performed with a simple cyclic interaction of repressors in *Escherichia coli*. Therefore, better understanding of cyclic gene regulatory network behaviors becomes the first key step to reveal the whole picture of large-scale gene regulatory networks.

Many works were devoted to understand the stability [1, 2, 22], robustness [14] and oscillatory behavior [11, 18] of cyclic gene regulatory networks. Regarding the oscillations, El-Samad *et al.* [18] and Hori *et al.* [11] recently provided analytic criteria for the existence of periodic oscillations. A key feature of their results is that the criteria are explicitly represented in terms of biochemical parameters, thus, one can easily observe the relation between the parameters and the periodic oscillations of protein concentrations. In fact, essential dimensionless quantities which are related to the existence of oscillations were found in [11].

In these previous works, however, the inherent time delays in transcription, translation and translocation process in gene regulatory networks were not considered in the model. Such time delays are essential especially for eukaryotic cells, because mRNA and protein productions occur at different locations in a cell, and the transportation of these substances results in sizable time delays [4]. Moreover, recent studies showed potential applications of the time delays as a feedback controller in gene regulatory networks [17, 23]. These facts motivate us to study the effect of time delays in cyclic gene regulatory networks.

The dynamical model of the gene regulatory networks with time delay was presented by Chen and Aihara [4]. Based on this model, several works analyzed the stability of equilibrium states of such genetic networks [6, 24, 25]. However, analytic criteria which enable us to gain biological insight on the relation between biochemical parameters and the dynamical behaviors of protein concentrations have not been obtained so far. Also, to the authors' knowledge, *oscillatory* behavior analysis has not been done for cyclic gene regulatory networks with time delay.

Therefore, the objective of this paper is to develop analytic criteria for the existence of oscillations in large-scale cyclic gene regulatory networks with sizable time delays, and reveal the effects of such delays based on the analytic result. The paper is organized as follows: In Section 2, we show that local instability of the unique equilibrium state results in oscillations of protein concentrations based on the Poincaré-Bendixson theorem for cyclic

time delay systems [16]. Then, Section 3 introduce an analytic scheme of local instability for large-scale linear time delay systems. In Section 4, we derive analytic conditions for the existence of oscillations, which can be applied to gene regulatory networks composed of arbitrary number of genes. The basic idea of our approach comes from framework of large-scale systems with a generalized frequency variable proposed by Hara *et al.* [8,21]. Section 5 is devoted to gain biological insights from the analytic conditions. In Section 6, our theorems are applied to two existing biological networks, Repressilator [5] and somitogenesis oscillator [15], with illustrative numerical simulations. Finally, Section 7 concludes the paper.

Some results in this paper were presented in our conference paper [20]. In this version of the paper, we further provide detailed mathematical proofs of the theorems and comparison with existing works. In particular, we show a counterexample of Theorem 2 in [4], which conflicts with our results, and argue the difference of the two results. We also present new examples of existing biological networks that make our theorems more convincing in Section 6.

2 Modelling and Nonlinear Analysis for Gene Regulatory Networks with Time Delay

2.1 Model of Cyclic Gene Regulatory Network with Time Delay

The well-known central dogma of molecular biology states that gene expression consists of the transcription and translation steps. During the transcription step, genes are decoded into molecules called messenger RNA (mRNA). Then, the information coded in mRNA is translated into proteins during the translation step. The rate of mRNA production is affected by the proteins called transcription factors, which are also created by the transcription and translation steps. Thus, there is an elaborate feedback mechanism to regulate protein levels in a cell as illustrated in Fig. 1. This networked system is called gene regulatory network.

In this paper, we consider the gene regulatory network, where each protein activates or represses another transcription in a cyclic way as depicted in Fig. 1. In particular, time delays arising from transportation and intermediate chemical reactions are explicitly considered. The dynamics of the cyclic gene regulatory network composed of N genes is modeled as

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

for $i = 1, 2, \dots, N$, where $r_i, p_i \in \mathbb{R}_+ (= \{x \in \mathbb{R} \mid x \geq 0\})$ denote the concentration of the i -th mRNA and its corresponding protein synthesized

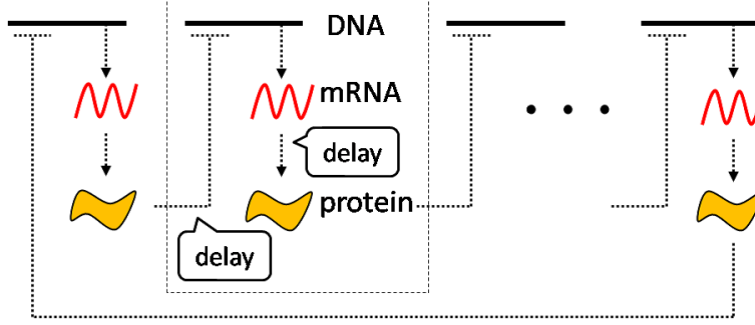


Figure 1: Cyclic gene regulatory network with time delay

in the i -th gene, respectively [4]. We define $p_0(t) := p_N(t)$ for the sake of notational simplification. Positive constants a_i, b_i, c_i and β_i have the following biological meanings: a_i and b_i denote the degradation rates of the i -th mRNA and protein, respectively; c_i and β_i denote the synthesis rates of the i -th mRNA and protein, respectively. A monotonic function $f_i : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ represents either repression or activation of the transcription. For repression, $f_i(\cdot)$ is defined as $f_i(\cdot) := f^-(\cdot)$ such that $f^-(0) = 1$ and $f^-(\infty) = 0$. For activation, $f_i(\cdot) := f^+(\cdot)$ such that $f^+(0) = 0$ and $f^+(\infty) = 1$. In this paper, we use the following Hill functions:

$$f^-(p) := \frac{1}{1 + p^\nu}, \quad f^+(p) := \frac{p^\nu}{1 + p^\nu}, \quad (2)$$

where $\nu(\geq 1)$ is a Hill coefficient, which represents the degree of cooperative binding and determines the degree of nonlinearity of the system. The constants τ_{r_i} and τ_{p_i} ($i = 1, 2, \dots, N$) represent time delays associated with the transcription and translation processes, respectively.

Let the following assumption be imposed throughout this paper:

Assumption 1.

$$\delta := \prod_{i=1}^N \delta_i < 0, \quad \text{where } \delta_i := \begin{cases} +1, & \text{for } f_i(\cdot) = f^+(\cdot), \\ -1, & \text{for } f_i(\cdot) = f^-(\cdot). \end{cases} \quad (3)$$

It is known that almost all solutions of (1) asymptotically converge to one of equilibria in the case of $\delta > 0$ [16]. Thus, it is reasonable to impose Assumption 1 to study the existence of oscillations, which is of our main interest in this paper.

2.2 Omega-limit Set of the System

The omega-limit set of the gene regulatory network system (1) can be specified by using a Poincaré-Bendixson type theorem for time delay systems

derived by Mallet-Paret and Sell [16]. The following proposition allows us to see that the omega-limit set of (1) is actually restricted to equilibrium points, periodic oscillations or homoclinic and heteroclinic orbits, and chaos is ruled out.

Proposition 1. [16] *Consider the following system.*

$$\begin{aligned}\dot{x}_i(t) &= g_i(x_i(t), x_{i+1}(t)), \quad (i=1, 2, \dots, n-1) \\ \dot{x}_n(t) &= g_n(x_n(t), x_1(t-1)),\end{aligned}\tag{4}$$

where $g_i(\cdot, \cdot)$ ($i=1, 2, \dots, n$) are C^1 nonlinear functions satisfying

$$z_i \frac{\partial g_i(w, v)}{\partial v} > 0 \quad \text{and} \quad z_i = \begin{cases} 1 & \text{if } i \neq n \\ z^* \in \{-1, 1\} & \text{if } i = n. \end{cases}\tag{5}$$

Let $\mathbf{x}(t) = [x_1(t), x_2(t), \dots, x_n(t)] \in \mathbb{R}^n$ be a solution of (4) on some interval $[t_0, \infty)$, and assume that $\mathbf{x}(t)$ is bounded in \mathbb{R}^n as $t \rightarrow \infty$. Then, the omega-limit set of $\mathbf{x}(t)$ consists of

- (a) a single non-constant periodic orbit,
- (b) equilibrium points, or
- (c) homoclinic and heteroclinic orbits.

The dynamical model of gene regulatory networks (1) can be transformed to the form (4) satisfying (5) by letting $n = 2N$ and x_i as follows.

$$\begin{cases} x_{2i-1}(t) = \sigma_{2i-1} p_{N-i+1}(Tt - \eta_{2i-1}), \\ x_{2i}(t) = \sigma_{2i} r_{N-i+1}(Tt - \eta_{2i}), \end{cases}\tag{6}$$

for $i = 1, 2, \dots, N$, where

$$T := \sum_{j=1}^{2N} \tau_j \quad \text{and} \quad \eta_i := \begin{cases} 0 & \text{for } i = 1 \\ \sum_{j=2}^i \tau_j & \text{for } i = 2, 3, \dots, 2N \end{cases}$$

with $\tau_{2i-1} := \tau_{p_{N-i+1}}$ and $\tau_{2i} := \tau_{r_{N-i+1}}$. The constants σ_i ($i = 1, 2, \dots, 2N$) take either $+1$ or -1 , and they are defined by

$$\sigma_i := \begin{cases} 1 & \text{for } i = 1 \\ \prod_{j=2}^i \rho_j & \text{for } i = 2, 3, \dots, 2N, \end{cases}$$

where

$$\begin{aligned}\rho_{2i-1} &:= \operatorname{sgn} \left[\frac{df_{N-i+2}}{dp} \right] \quad \text{for } i = 1, 2, \dots, N. \\ \rho_{2i} &:= 1\end{aligned}$$

The constant z^* is then determined as $z^* = \prod_{i=1}^{2N} \rho_i$. The detailed proof is provided in Appendix A. Note that the above transformation affects only

the sign of the omega-limit set, thus, the omega-limit set of $r_i(t)$ and $p_i(t)$ can be specified, once that of $x_i(t)$ is obtained.

Boundedness of $x(t)$ is easily verified in the similar way to El-Samad *et al.* [18], where non-delay cyclic gene regulatory networks were considered. Existence of an equilibrium point and its uniqueness were proved in Hori *et al.* [11] in the case of no time delay, and time delay does not affect these properties of the equilibrium point. Hence, the following lemma readily follows from Proposition 1.

Lemma 1. *Consider the cyclic gene regulatory networks modeled by (1). Then, the protein levels $p_i(t)$ ($i = 1, 2, \dots, N$) exhibit (a) non-constant periodic oscillations, (b) convergence to the equilibrium point, or (c) homoclinic orbits.*

Note that chaos is ruled out for the system (1). This lemma immediately leads to the following proposition, which becomes a key to deriving existence conditions of oscillations in Section 4.

Proposition 2. *Consider the cyclic gene regulatory networks modeled by (1). The system has oscillations of protein levels $p_i(t)$ ($i = 1, 2, \dots, N$), if the unique equilibrium point is locally unstable.*

In this paper, the term 'oscillations' refers to both non-constant periodic and homoclinic orbits. It should be noted that oscillations are periodic except for the case of homoclinic orbits.

3 Stability Analysis of the Linearized Model

We see from Proposition 2 that the local instability at a unique equilibrium point implies the existence of oscillations. Hence, a linearized model of gene regulatory networks is introduced in this section, then a simple graphical test for local stability analysis is presented.

3.1 Linearized Model of Gene Regulatory Networks

Consider a linearized system of (1) at the unique equilibrium point $[r_1^*, p_1^*, \dots, r_N^*, p_N^*]^T$. Let $\hat{r}_i(s)$ and $\hat{p}_i(s)$ denote Laplace transform of $r_i(t)$ and $p_i(t)$, respectively. Then, Laplace transform of the linearized system with zero initial condition is obtained for $i = 1, 2, \dots, N$ as

$$\begin{bmatrix} s\hat{r}_i(s) \\ s\hat{p}_i(s) \end{bmatrix} = \begin{bmatrix} -a_i & 0 \\ c_i e^{-s\tau_{r_i}} & -b_i \end{bmatrix} \begin{bmatrix} \hat{r}_i(s) \\ \hat{p}_i(s) \end{bmatrix} + \begin{bmatrix} \beta_i e^{-s\tau_{p_{i-1}}} \\ 0 \end{bmatrix} \hat{u}_i(s),$$

where $\hat{u}_i(s) := \zeta_i \hat{p}_{i-1}(s)$ and $\zeta_i := f'_i(p_{i-1}^*)$. Thus, the transfer function of the i -th gene from \hat{u}_i to \hat{p}_i denoted by $h_i(s)$ is computed as

$$h_i(s) = \frac{R_i^2 e^{-s(\tau_{r_i} + \tau_{p_{i-1}})}}{(T_{r_i}s + 1)(T_{p_i}s + 1)}, \quad (7)$$

where

$$R_i := \frac{\sqrt{c_i \beta_i}}{\sqrt{a_i b_i}}, \quad T_{r_i} := \frac{1}{a_i}, \quad T_{p_i} := \frac{1}{b_i}. \quad (8)$$

The constant R_i represents the ratio of the geometric means of synthesis rates and degradation rates of the i -th gene, and it is known as one of the essential biological quantities which characterize the oscillations in gene regulatory networks [11]. The cyclic gene regulatory network system can be considered as the cyclic interconnection of the dynamical system $h_i(s)$ ($i = 1, 2, \dots, N$).

Let the following assumption on the degradation rates be imposed throughout this paper.

Assumption 2. $a_1 = a_2 = \dots = a_N (= a)$ and $b_1 = b_2 = \dots = b_N (= b)$, *i.e.*, mRNAs and proteins have common degradation rates between genes.

Then, the overall system can be written by a transfer function $\mathcal{H}(s)$ defined by

$$\mathcal{H}(s) := (\phi(s)e^{s\tau}I - K)^{-1}, \quad \phi(s) := \frac{1}{h(s)}, \quad (9)$$

where

$$h(s) := \frac{1}{(T_r s + 1)(T_p s + 1)}, \quad T_r := \frac{1}{a}, T_p := \frac{1}{b}, \quad (10)$$

$$\tau := \frac{1}{N} \sum_{i=1}^N (\tau_{r_i} + \tau_{p_i}), \quad (11)$$

$$K := \begin{bmatrix} 0 & 0 & \dots & 0 & \zeta_1 R_1^2 \\ \zeta_2 R_2^2 & 0 & \dots & 0 & 0 \\ 0 & \zeta_3 R_3^2 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & \zeta_N R_N^2 & 0 \end{bmatrix}. \quad (12)$$

The time delays τ_{r_i} and τ_{p_i} ($i = 1, 2, \dots, N$) of $h_i(s)$ can be different between genes, but the cyclic structure and the distributive property of linear systems allows us to equally distribute the time delays over all genes. Thus, the system $\mathcal{H}(s)$ shares the time delay τ among all genes, where τ represents the average of time delays in the network. Note that the structure of the matrix K is determined from the graph topology of the gene regulatory network, and $\phi(s)e^{s\tau}$ is determined from the dynamics of each gene's expression. In the next section, we will show that the stability of $\mathcal{H}(s)$ can be systematically analyzed using a simple graphical condition which is easy to apply even for large-scale systems.

3.2 Graphical Condition for Local Instability

In this subsection, we provide a systematic way to determine the local instability of the large-scale time delay system $\mathcal{H}(s)$. In particular, a necessary and sufficient graphical condition for local instability is presented for the cyclic gene regulatory networks consisting of any number of genes.

First, we introduce an instability region of the large-scale linear system $\mathcal{H}(s)$, which is characterized by the gene's dynamics $h(s)e^{-s\tau}$. Let a set of complex values Ω_+ be defined as

$$\Omega_+ := \{\lambda \in \mathbb{C} \mid \exists s \in \mathbb{C}_+, \phi(s)e^{s\tau} = \lambda\}, \quad (13)$$

where $\mathbb{C}_+ := \{s \in \mathbb{C} \mid \text{Re}[s] > 0\}$. The set Ω_+ is the image of the open right-half complex plane under the mapping $\phi(s)e^{s\tau}$. The instability of $\mathcal{H}(s)$ can be characterized by Ω_+ and the matrix K as follows.

Proposition 3. *Consider the system $\mathcal{H}(s)$ defined by (9). Then, at least one pole of $\mathcal{H}(s)$ lies in the open right half plane of the complex plane, if and only if*

$$\text{spec}(K) \cap \Omega_+ \neq \emptyset. \quad (14)$$

The idea behind this proposition is that the mapping $\phi(s)e^{s\tau}$ can be considered as a generalized frequency variable proposed in [8, 21], since $\mathcal{H}(s)$ in (9) is obtained by replacing the frequency variable 's' of the transfer function $(sI - K)^{-1}$ with $\phi(s)e^{s\tau}$. Thus, the above graphical criterion is obtained in the same way as Proposition 5.1 in Hara *et al.* [8], though time delay was not considered in the previous work. An example of the instability region Ω_+ is illustrated in Fig. 2.

The stability counterpart of Proposition 3 is characterized by the set $\Omega_+^c := \{\lambda \in \mathbb{C} \mid \forall s \in \bar{\mathbb{C}}_+, \phi(s)e^{s\tau} \neq \lambda\}$ with $\bar{\mathbb{C}}_+ := \{s \in \mathbb{C} \mid \text{Re}[s] \geq 0\}$, which is an open complementary set of Ω_+ . Then, it follows that all the eigenvalues of K lie in the stability region Ω_+^c if and only if $\mathcal{H}(s)$ is asymptotically stable.

Remark 1. Regarding the necessary and sufficient stability condition of $\mathcal{H}(s)$, Chen and Aihara [4] presented a similar graphical test (see Theorem 2 in [4]). The authors, however, have found that their graphical test is incorrect. A counterexample to their result is shown below.

Let $N = 3, a = b = 1.0000, c_i = \beta_i = 1.7498, \nu = 2.0000$ and $\tau_{r_i} = \tau_{p_i} = 0.5000$ ($i = 1, 2, 3$). Then, it follows that $\phi(s)e^{s\tau} = (s + 1)^2 e^{0.5s}$, $R_1^2 = R_2^2 = R_3^2 = 1.7498$ and $\zeta_1 = \zeta_2 = \zeta_3 = 0.6858$. Theorem 2 in [4] states that the system $\mathcal{H}(s)$ is stable if and only if all the eigenvalues of the matrix K lie inside the region specified by an Archimedean spiral illustrated

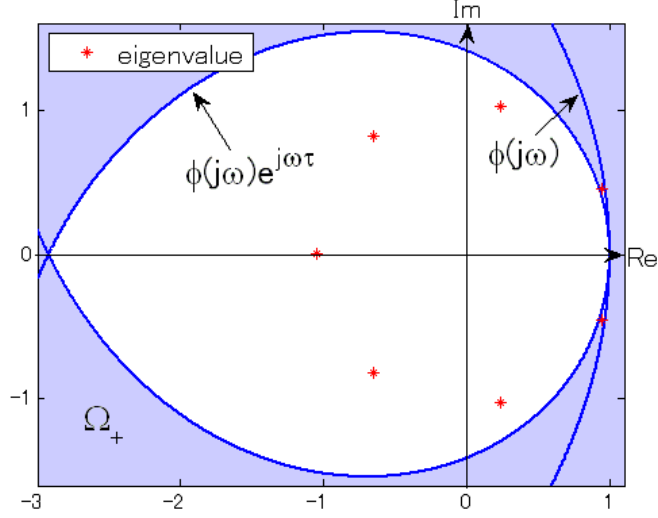


Figure 2: The instability region Ω_+ and the eigenvalue locations of K .

in Fig. 7(a) (see Appendix B for details). The eigenvalues of the matrix K are obtained as $\{1.2e^{\pm j\pi/3}, -1.2\}$, and $\mathcal{H}(s)$ would be concluded as stable from Fig. 7(a) in Appendix B.

The Nyquist plot of the loop transfer function $\mathcal{H}(s)$, however, verifies that it is actually unstable as shown in Fig. 7(b). Also, the numerical simulation of the protein time course starts oscillations as shown in Fig. 8. Therefore, Theorem 2 in [4] is not the necessary and sufficient stability condition for $\mathcal{H}(s)$. We point out errors in their mathematical proof in Appendix B.

It should be noted that the stability region Ω_+^c is illustrated in Fig. 7(a), and Proposition 3 in this paper concludes that $\mathcal{H}(s)$ is unstable in the above example. \square

Next, we provide some characterizations of stability of $\mathcal{H}(s)$ derived from the graphical test in Proposition 3. Consider the matrix K in (12). A key feature of this matrix is that the eigenvalues are equally distributed on a circle with the center at the origin. Specifically, the eigenvalues of K are written as

$$\lambda_k := L e^{\frac{j(2k-1)\pi}{N}} \quad (k = 1, 2, \dots, N) \quad (15)$$

with

$$L := \prod_{i=1}^N |\zeta_i R_i^2|^{\frac{1}{N}}. \quad (16)$$

Note that L is the radius of the circle. We designate L as the average gain, since it is a geometric mean of the feedback gains of K in (12). These features lead to the following characterization of the instability of $\mathcal{H}(s)$.

Lemma 2. *Consider the system $\mathcal{H}(s)$ defined by (9). Then, the following conditions are equivalent.*

- (i) $\exists k \lambda_k \in \Omega_+$.
- (ii) $\lambda_1 \in \Omega_+$.
- (iii) $\exists \omega_{\#}$ such that $|\phi(j\omega_{\#})e^{j\omega_{\#}\tau}| < L$ and $\arg(\phi(j\omega_{\#})e^{j\omega_{\#}\tau}) = \pi/N$.
- (iv) $\exists \omega_*$ such that $\arg(\phi(j\omega_*)e^{j\omega_*\tau}) > \pi/N$ and $|\phi(j\omega_*)e^{j\omega_*\tau}| = L$.

where $\arg(\cdot)$ is the argument of a complex number.

Proof. (i) \Leftrightarrow (ii): The proof is mainly based on the fact that both $|\phi(j\omega)e^{j\omega\tau}|$ and $\arg(\phi(j\omega)e^{j\omega\tau})$ monotonically increase for positive ω . The monotonicity is obvious from the definition (9). Then, it is easily verified that λ_1 , which is the eigenvalue closest to the positive real axis, always goes inside the region Ω_+ first, since the eigenvalues of the matrix K are located on a circle center at the origin and radius L (see Fig. 2).

(ii) \Leftrightarrow (iii): Let $\omega_{\#}$ denote a frequency such that $\arg(\phi(j\omega_{\#})e^{j\omega_{\#}\tau}) = \pi/N$. The conclusion immediately follows from the fact that $|\lambda_1| = L$ and $\arg(\lambda_1) = \pi/N$.

(iii) \Leftrightarrow (iv): We only show (iii) \Rightarrow (iv), since the converse can be shown in a similar manner. Suppose (iii) is satisfied. Let ω_* be defined such that $|\phi(j\omega_*)e^{j\omega_*\tau}| = L$. It follows that $\omega_{\#} < \omega_*$, because $|\phi(j\omega_{\#})e^{j\omega_{\#}\tau}| < |\phi(j\omega_*)e^{j\omega_*\tau}| = L$ and there is the gain monotonicity for $\phi(j\omega)e^{j\omega\tau}$ as shown above. Then, the phase monotonicity implies $\arg(\phi(j\omega_{\#})e^{j\omega_{\#}\tau}) = \pi/N < \arg(\phi(j\omega_*)e^{j\omega_*\tau})$. \square

The condition (ii) in Lemma 2 greatly simplifies the graphical test, because the instability can be determined by the position of one specific eigenvalue λ_1 and the region Ω_+ . The condition (iii) is an analytic version of the consequence (ii), though it is generally difficult to obtain $\omega_{\#}$ in terms of the system's parameters. In the next section, the condition (iv) plays a key role to derive the analytic conditions for the existence of oscillations.

4 Existence Conditions of Oscillations

In this section, we provide analytic existence conditions of oscillations.

4.1 Existence Conditions of Oscillations in terms of Average Gain

Recall that local instability of $\mathcal{H}(s)$ is a sufficient condition for the existence of oscillations as shown in Proposition 2, and the instability of $\mathcal{H}(s)$ can be examined by the graphical test presented in Proposition 3. The following graphical condition is a direct consequence of these results.

Proposition 4. *Consider the cyclic gene regulatory networks modeled by (1). Suppose Assumptions 1 and 2 hold. Then, the system has oscillations of protein concentrations $p_i(t)$ ($i = 1, 2, \dots, N$), if*

$$\text{spec}(K) \cap \Omega_+ \neq \emptyset. \quad (17)$$

Note that (17) is the same graphical condition as the one in Proposition 3.

In what follows, analytic conditions are derived based on this graphical condition. We first introduce normalized parameters of gene regulatory networks to avoid notational complexity, and to capture the essence of mathematical conditions. Let T_A and T_G denote the arithmetic and geometric means of the mRNA and protein degradation time constants, *i.e.*,

$$T_A := \frac{T_r + T_p}{2}, \quad T_G := \sqrt{T_r T_p}. \quad (18)$$

The constants T_A and T_G have the physical dimension of time. Define the following dimensionless constants $Q, \tilde{\omega}$ and $\tilde{\tau}$,

$$Q := \frac{T_G}{T_A}, \quad \tilde{\omega} := \omega T_A, \quad \tilde{\tau} := \frac{\tau}{T_A}. \quad (19)$$

Then, the boundary of the region Ω_+ defined in (13) can be written as

$$\phi(j\omega)e^{j\omega\tau} = (-Q^2\tilde{\omega}^2 + 1 + 2j\tilde{\omega})e^{j\tilde{\omega}\tilde{\tau}}. \quad (20)$$

We see that the eigenvalues of K and the region Ω_+ are characterized in analytic form as (15) and (20), respectively. This leads to analytic conditions for the existence of oscillations. We first show existence conditions in terms of the average gain L in (15).

Theorem 1. *Consider the gene regulatory networks modeled by (1). Suppose Assumptions 1 and 2 hold. Define the two functions $W(N, Q)$ and $D(Q, L)$ as*

$$W(N, Q) := \frac{2}{\cos \frac{\pi}{N} + \sqrt{\cos^2 \frac{\pi}{N} + Q^2 \sin^2 \frac{\pi}{N}}}, \quad (21)$$

$$D(Q, L) := 4(1 - Q^2) + Q^4 L^2. \quad (22)$$

Then, the system has oscillations of protein concentrations $p_i(t)$ ($i = 1, 2, \dots, N$), if one of the following two conditions holds ¹.

(i) $L > W(N, Q)$,

(ii) $1 < L \leq W(N, Q)$ and

$$\begin{aligned} \arg \left(2 - \sqrt{D(Q, L)} + j2\sqrt{Q^2 - 2 + \sqrt{D(Q, L)}} \right) \\ > \frac{\pi}{N} - \frac{\sqrt{Q^2 - 2 + \sqrt{D(Q, L)}}}{Q^2} \tilde{\tau}. \end{aligned} \quad (23)$$

Proof. It follows from Proposition 2 that there exists oscillations if the unique equilibrium point of (1) is unstable. Hence, we consider the instability condition of $\mathcal{H}(s)$, for which the simple graphical test in Proposition 3 is available.

We first consider the case of $L \leq 1$. It should be noted that the average gain L is the radius of the circle where eigenvalues are located. It follows that $L \leq 1 \leq |\phi(j\omega)e^{j\omega\tau}|$ for all ω . Since $|\phi(j\omega)e^{j\omega\tau}| = 1$ only when $\omega = 0$, and $\arg(\phi(j\omega)e^{j\omega\tau}) = 0$ for $\omega = 0$, there is no ω_* satisfying the condition (iv) in Lemma 2. Thus, Lemma 2 implies $\lambda_1 \notin \Omega_+$, and $\mathcal{H}(s)$ is not unstable.

In the case of $L > W(N, Q)$, we readily see $\lambda_1 \in \Omega_+$ according to Theorem 2 in [11]. In the case of $1 < L \leq W(N, Q)$, consider the condition (iv) in Lemma 2. Then, $|\phi(j\omega_*)e^{j\omega_*\tau}| = L$ yields

$$Q^4 \tilde{\omega}_*^4 + 2(2 - Q^2) \tilde{\omega}_*^2 + 1 - L^2 = 0, \quad (24)$$

where $\tilde{\omega}_* := \omega_* T_A$. Then, $\tilde{\omega}_*$ is obtained as

$$\tilde{\omega}_* = \frac{\sqrt{Q^2 - 2 + \sqrt{D(Q, L)}}}{Q^2}, \quad (25)$$

and (23) is derived by substituting $\tilde{\omega}_*$ into $\arg(\phi(j\omega_*)e^{j\omega_*\tau}) > \pi/N$. \square

The existence of oscillations can be determined by substituting the given parameters into Theorem 1. In particular, biological insights can be obtained by observing the relation between the quantities, which will be introduced in Section 5.

Since (23) has a certain monotone property in terms of L , we can simplify Theorem 1, and obtain the equivalent condition as follows.

Corollary 1. *Consider the cyclic gene regulatory networks modeled by (1) Suppose Assumptions 1 and 2 hold. Define $W(N, Q)$ and $D(Q, L)$ as*

¹This condition is necessary and sufficient for local instability of $\mathcal{H}(s)$, which is readily seen from the proof.

(21) and (22), respectively. Then, the system has oscillations of protein concentrations $p_i(t)$ ($i = 1, 2, \dots, N$), if $L > \bar{L}$, where \bar{L} is the solution of the equation

$$\begin{aligned} \arg \left(2 - \sqrt{D(Q, \bar{L})} + j2\sqrt{Q^2 - 2 + \sqrt{D(Q, \bar{L})}} \right) \\ = \frac{\pi}{N} - \frac{\sqrt{Q^2 - 2 + \sqrt{D(Q, \bar{L})}}}{Q^2} \tilde{\tau}. \end{aligned} \quad (26)$$

In particular, the solution \bar{L} is uniquely determined and satisfies $\bar{L} \in (1, W(N, Q)]$.

The proof of Corollary 1 is attached in Appendix C.

Remark 2. In the case of $\tau = 0$, Theorem 1 and Corollary 1 coincide with Theorem 2 in [11], which provides an existence condition of periodic oscillations for non-delay case.

Remark 3. \bar{L} is a monotonically decreasing function in terms of N, Q , and $\tilde{\tau}$. This fact implies that the system tends to have oscillations as

- the number of genes, N gets larger,
- normalized time delay, $\tilde{\tau}$, gets larger, and
- Q approaches to unity.

Note that Q is the ratio of the arithmetic and geometric means of degradation rates, thus $Q \rightarrow 1$ means that the mRNA and protein degradation rates get closer.

4.2 Existence Conditions of Oscillations in terms of Biological Parameters

We have obtained the conditions for the existence of oscillations in terms of the average gain. In Theorems 1 and Corollary 1, the value of L depends on ζ_i , which is determined from the equilibrium point p_i^* . Since the equilibrium point depends on the parameters a, b, c_i and β_i , Theorem 1 and Corollary 1 require a numerical computation of the equilibrium point to determine ζ_i . It is, however, desirable to explicitly take its dependence into account in the analytic conditions in order to gain biological insights on the relation between the parameters and the existence of oscillations. In this section, we restrict our attention to a class of the cyclic gene regulatory networks, and present analytic conditions for the existence of oscillations that explicitly consider the dependence of the equilibrium on the parameters.

We consider the class of systems that satisfy $f_i(\cdot) = f^-(\cdot)$ for all $i = 1, 2, \dots, N$, and that $R_1 = R_2 = \dots = R_N (= R)$ in this section. Note that this class of regulatory networks includes Repressilator [5].

We first see that the equilibrium p_i^* does not change by time delay, and $p_1^* = p_2^* = \dots = p_N^* (= p^*)$ and $\zeta_1^* = \zeta_2^* = \dots = \zeta_N^* (= \zeta^*)$ hold as shown in [10]. Using this property, we have the following relation between L and R .

Lemma 3. *Consider the gene regulatory networks modeled by (1). Suppose $f_i(\cdot) = f^-(\cdot)$ ($i = 1, 2, \dots, N$), $R_1 = R_2 = \dots = R_N (= R)$, and Assumptions 1 and 2 hold. Then $L < \nu$, and the following equality holds.*

$$R^2 = \left(\frac{L}{\nu - L} \right)^{1/\nu} \frac{\nu}{\nu - L}. \quad (27)$$

Proof. It follows that

$$L = -R^2 \zeta = -R^2 \left(\frac{-\nu p^{*\nu-1}}{(1 + p^{*\nu})^2} \right), \quad (28)$$

where the second equality follows from the definition of ζ . According to [10], we have

$$p^* = \frac{R^2}{1 + p^{*\nu}}. \quad (29)$$

Then, it follows from (28) and (29) that

$$L = \frac{\nu p^{*\nu}}{1 + p^{*\nu}}. \quad (30)$$

This implies $L < \nu$. In addition, it follows that

$$\zeta = -\frac{\nu}{R^4} (R^2 - p^*) \quad (31)$$

(see [10] for the details). Multiplicating R^2 to (31), we have

$$L = -\frac{\nu}{R^2} (R^2 - p^*). \quad (32)$$

Thus, we can eliminate p^* from (30) by using (32), and obtain (27). \square

Lemma 3 shows a direct relation between the average gain L and the biological parameters, R and ν . Then, this lemma leads to the following existence condition of oscillations, which is explicitly written in terms of the biological parameters.

Theorem 2. *Consider the gene regulatory networks modeled by (1). Suppose $f_i(p) = f^-(p)$ ($i = 1, 2, \dots, N$), $R_1 = R_2 = \dots = R_N (= R)$, and*

Assumptions 1 and 2 hold. Define $W(N, Q)$, $D(Q, L)$ and \bar{L} as (21), (22) and (26), respectively. Then, the system has oscillations of protein concentrations $p_i(t)$ ($i = 1, 2, \dots, N$) if both $\nu > \bar{L}$ and $R > \bar{R}$ hold, where

$$\bar{R}^2 := \left(\frac{\bar{L}}{\nu - \bar{L}} \right)^{1/\nu} \frac{\nu}{\nu - \bar{L}}. \quad (33)$$

Proof. We derive an equivalent condition to Corollary 1. Observe that R^2 in (27) is monotonically increasing for $L(< \nu)$. Thus, if the condition $\bar{L} < L$ in Corollary 1 is satisfied, $\bar{R} < R$ follows, where \bar{R}^2 is defined by (33). We see from Lemma 3 that $\nu > \bar{L}$ is also satisfied, because $\nu > L$. On the other hand, if $\nu > \bar{L}$ and $R > \bar{R}$ are satisfied, we have $L > \bar{L}$ because of the monotonicity of (27).

Since the conditions $\nu > \bar{L}$ and $R > \bar{R}$ are equivalent to those of Corollary 1, we can conclude that there exists oscillations if these conditions are satisfied. \square

Theorem 2 provides a condition for the existence of oscillations in terms of biological parameters ν , R and $\bar{R}(\nu, \bar{L}(N, Q, \tilde{\tau}))$ without any information about the equilibrium point p^* . This is contrast with the conditions in Theorem 1 and Corollary 1. Therefore, the essential parameters related to the existence of oscillations are the following five parameters: the number of genes (N), time delay normalized by the arithmetic mean of the lifetime ($\tilde{\tau}$), the Hill coefficient (ν), the ratio between the geometric mean of degradation rate and production rates (R), and the ratio between the geometric and arithmetic means of degradation rates (Q).

Remark 4. We can see that \bar{R} and \bar{L} are monotone with respect to the system's parameters. This observation leads to the conclusion that the system tends to have oscillations by increasing any of the above five essential quantities, N , $\tilde{\tau}$, ν , R and Q .

5 Biological Consideration

In this section, the results presented in the previous section are biologically interpreted with illustrative numerical simulations.

5.1 Effects of Time Delay on the Existence of Oscillations

Consider the cyclic gene regulatory networks composed of $N = 7$ genes. Assume that $a = 1.2$, $b = 4.8$, $c_1 = c_3 = c_6 = c_7 = 1.92$, $c_2 = c_4 = c_5 = 3.84$, $\beta_1 = \beta_3 = \beta_6 = \beta_7 = 4.32$, $\beta_2 = \beta_4 = \beta_5 = 2.16$, and let the Hill function be defined as $f_i(p) = 1/(1 + p^\nu)$ with $\nu = 2.6$ for $i = 1, 2, \dots, N$. Then, Q and R ($:= R_1 = R_2 = \dots = R_7$) are obtained as $Q = 0.800$ and

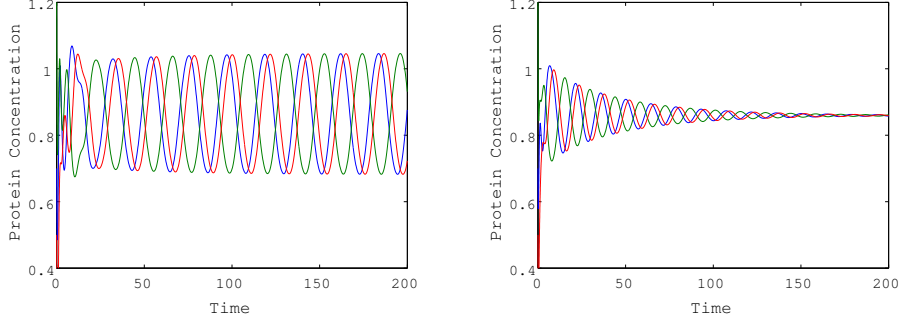
$R = 1.200$ from the definition (19) and (8), respectively. The value of L in (15) can be computed as $L = 1.048$. Note that ζ_i in L involves computation of the unique equilibrium of the system, but it can be efficiently done with the bisection algorithm (see [10] for details). In the following, we will see the effect of time delay by comparing a genetic regulatory network with and without time delay.

We first apply the graphical existence condition in Proposition 4. Figure 2 illustrates the instability region Ω_+ and the eigenvalue distribution of K for $\tilde{\tau} = 0$ and $\tilde{\tau} = 1.00$, where the time delays of the reactions are set as $\tau_{r_1} = \tau_{r_3} = \tau_{r_4} = \tau_{r_7} = 0.31, \tau_{r_2} = \tau_{r_5} = \tau_{r_6} = 0.26, \tau_{p_1} = \tau_{p_3} = \tau_{p_4} = \tau_{p_7} = 0.21, \tau_{p_2} = \tau_{p_5} = \tau_{p_6} = 0.26$, thus the average of the time delay is $\tau = 0.52$. In the case of $\tilde{\tau} = 1.00$, the boundary of the instability region Ω_+ is given by $\phi(j\omega)e^{j\omega\tau}$ in Fig. 2. Thus, Proposition 4 implies the existence of oscillations, because two eigenvalues of K belong to the region Ω_+ . In the case of $\tilde{\tau} = 0$, the boundary of the instability region Ω_+ is $\phi(j\omega)$ in Fig. 2. We can see that all eigenvalues of K are located outside the region Ω_+ when $\tilde{\tau} = 0$. Thus, it is concluded from Proposition 3 that a unique equilibrium point of the system is locally asymptotically stable, and the protein concentrations do not exhibit oscillations when they are perturbed around the equilibrium point. Note that this result does not imply non-existence of oscillations, since Proposition 4 is a sufficient condition for the existence of oscillations.

The same conclusion follows from the analytic conditions in Theorem 1. We can see from Theorem 1 that there exist oscillations when $\tilde{\tau} = 1.00$, because $L = 1.048 > \bar{L} = 1.031$, where \bar{L} is computed by (26). On the other hand, $L = 1.048 < \bar{L} = 1.072$ in the case of $\tilde{\tau} = 0$. Since the condition in Theorem 1 is equivalent to that of Proposition 3, we can conclude that the equilibrium point is locally asymptotically stable.

Theorem 1 and Corollary 1 required the value of equilibrium point to compute L . In contrast, Theorem 2 does not require the computation of the equilibrium. For given parameters, \bar{L} and \bar{R} can be determined from (26) and (33), respectively. Specifically, $\bar{L} = 1.031$ and $\bar{R} = 1.187$ when $\tilde{\tau} = 1.00$. Computing R from (8) yields $R = 1.200$. Therefore, both of $\nu = 2.6 > \bar{L} = 1.031$ and $R = 1.200 > \bar{R} = 1.187$ in Theorem 2 are satisfied, and the existence of oscillations is concluded. On the other hand, $\bar{R} = 1.218$ when $\tilde{\tau} = 0$. Thus, the conditions in Theorem 2 do not hold, because $R = 1.200 \leq \bar{R} = 1.218$ despite $\nu = 2.6 > \bar{L} = 1.072$. It should be noted that this result, in turn, implies that there exists oscillations even for $\tilde{\tau} = 0$, if the parameters a, b, c_i and β_i are set so that $R > 1.218$ and $\bar{L} < 2.6$.

In fact, numerical simulations shown in Fig. 3 show oscillations and convergence to the equilibrium of protein concentrations for the time delay and non-delay case, respectively. We have seen that the existence of oscillations is more probable when the time delay is large. In what follows, we will see



(a) Case: $\tilde{\tau} = 1$. The protein concentrations exhibit oscillations.

(b) Case: $\tilde{\tau} = 0$. The protein concentrations converge to the equilibrium.

Figure 3: Time plot of protein concentrations.

that this is indeed the case in general.

5.2 Effects of Parameters on the Existence of Oscillations

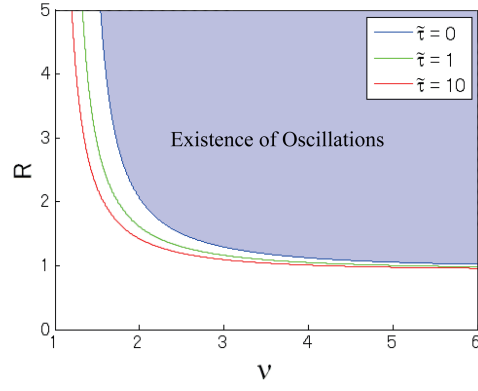
As we have seen in Theorem 2, the existence of oscillations in cyclic gene regulatory networks with time delay can be characterized by the five dimensionless parameters $N, Q, \tilde{\tau}, \nu$ and R . The parameter regions that guarantee the existence of oscillations can be drawn as shown in Fig. 4 based on the analytic conditions given in Theorem 2. From these figures, we can readily conclude that the system tends to have oscillations as ν and R get larger. In addition, the larger the parameters $\tilde{\tau}, Q$ and N are, the more probable the existence of oscillations becomes because of each figure in Fig. 4.

An advantage of Theorem 2 is that we can confirm that these observations are true in general because the conditions are written in an analytic form in terms of the given biological parameters. Therefore, we conclude that the gene regulatory networks, in general, tends to have oscillations by letting the five essential parameters $N, Q, \tilde{\tau}, \nu$ and R larger (see also Remark 4)².

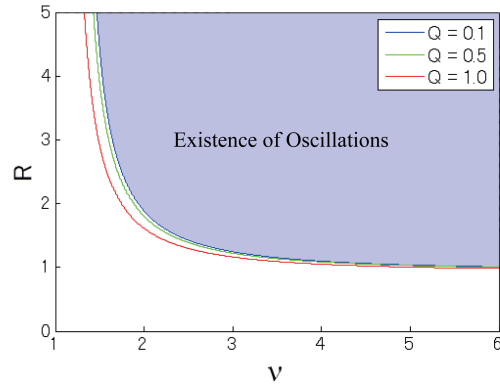
6 Applications

In this section, we will apply our results to two existing biological systems, and see how our results work in analyzing the effect of time delay on the existence of oscillations.

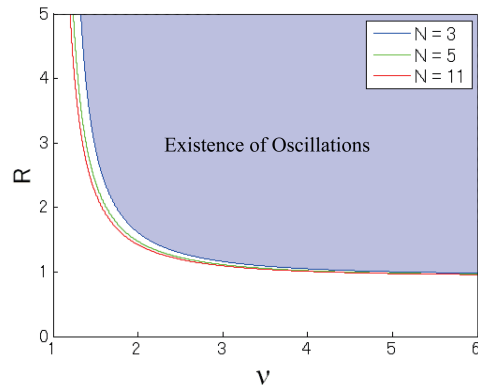
²The constant \bar{R} in Theorem 2 is not monotone decreasing for all $\nu (\geq 1)$, but it becomes a decreasing function for $1 \leq \nu \leq 8$, which is the region of our interest.



(a) $\tilde{\tau} = 0, 1$ and 10 with $N = 3$ and $Q = 1$



(b) $Q = 0.1, 0.5$ and 1.0 with $N = 3$ and $\tilde{\tau} = 1$



(c) $N = 3, 5$ and 11 with $Q = 1$ and $\tilde{\tau} = 1$

Figure 4: Parameter regions (ν, R) for the existence of oscillations.

6.1 Repressilator

Repressilator is one of the pioneering synthetic gene regulatory networks created by Elowitz and Leibler [5]. This artificial cyclic gene regulatory network is composed of three repressor genes, each of which represses another gene and forms cyclic reaction structure shown in Fig. 1. In [5], Repressilator was implemented in *Escherichia Coli*, and oscillations of protein concentrations were observed in vitro.

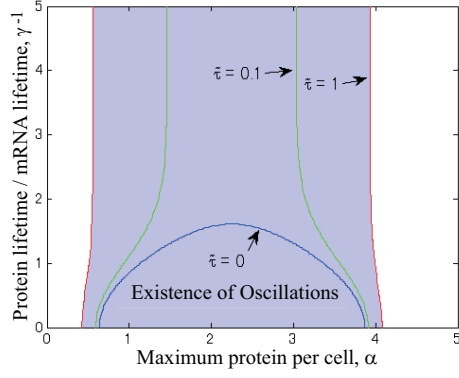
The dynamical model of Repressilator can be written as

$$\begin{cases} \dot{r}_i(t) = -r_i(t) + \frac{\alpha}{1 + p_{i-1}(t - \tau_{r_{i-1}})^\nu} + \alpha_0, \\ \dot{p}_i(t) = -\gamma(p_i(t) - r_i(t - \tau_{r_i})), \end{cases} \quad (34)$$

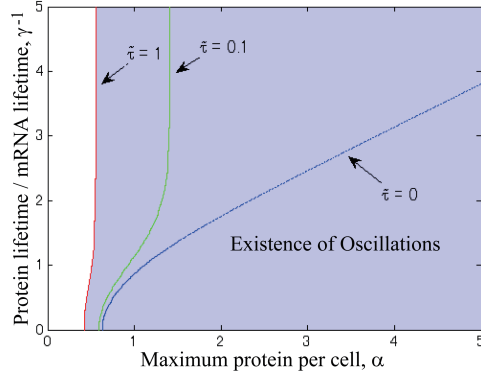
for $i = 1, 2, 3$, where γ denotes the ratio of the protein degradation rate to the mRNA degradation rate, and the constant α_0 represents leakiness of the promoter [5]. Note that time delays are not considered, *i.e.*, $\tau_{r_i} = \tau_{p_i} = 0$ for $i = 1, 2, 3$, in the original paper [5]. We remark that the recently proposed technique [23] could enable us to engineer the time delay, and it would contribute to obtaining a desired dynamics (see, for example, [17]). Hence, the time delays in the above model should account for such engineered delay as well as fast dynamics omitted in the modelling process. It can be seen that the model (34) is equivalent to (1) by rescaling the parameters when $\alpha_0 = 0$. We notice that Proposition 2 holds, even when $\alpha_0 \neq 0$, and Theorem 1 and Corollary 1 can be applied to analyze the existence of oscillations.

Let us first consider the case where no time delay appears in dynamics, *i.e.*, $\tau_{r_i} = \tau_{p_i} = 0$ ($i = 1, 2, \dots, N$), which is the original model of Repressilator presented in [5]. Following the numerical simulations conducted in [5], we set $\alpha = 624, \alpha_0 = 0.0866, \beta = 0.200$ and $\nu = 2.0$. Then, L and \bar{L} in Corollary 1 can be computed as $L = 1.833$ and $\bar{L} = 1.519$, respectively. Thus, we conclude the existence of oscillations from Corollary 1, which is consistent with the simulation result in [5].

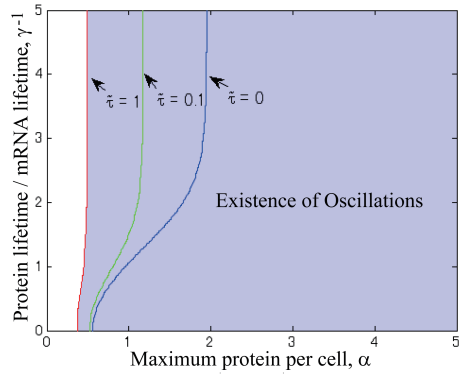
Next, we investigate the effect of time delay on the existence of oscillations, and show that time delay increases robustness of Repressilator. Here we numerically examined the existence conditions in Theorem 1 for various time delays and parameters. The result is shown in Fig. 5, where the parameter regions for the existence of oscillations are illustrated. Note that only the normalized time delay $\tilde{\tau}$ rather than each time delay itself affects the existence of oscillations as seen in Section 5.2. Also note that the parameter region for $\tilde{\tau} = 0$ in Fig. 5 coincides with that in Fig. 1 (b) in [5]. We can see from Fig. 5 that the regions for the existence of oscillations get larger as $\tilde{\tau}$ become larger. This implies that one could make robust oscillator by inserting time delay. Moreover, the parameter region is not sensitive to a little change of α_0 and ν when $\tilde{\tau}$ is large.



(a) $\alpha_0 = 10^{-3}\alpha, \nu = 2$



(b) $\alpha_0 = 0, \nu = 2$



(c) $\alpha_0 = 0, \nu = 2.1$

Figure 5: Parameter regions (α, γ^{-1}) for the existence of oscillations in Repressilator. Both axes are in common log scale. Oscillations are more probable as the time delay becomes large.

6.2 Somitogenesis Oscillator

Somitogenesis is the process by which the somites of living organisms are created. Biological experiments as well as theoretical studies showed that the timing of the somite segmentation is regulated by an oscillatory expression of *Hes7* gene (see [9, 15] and the references therein). In particular, it was shown by a biological experiment that oscillations produced by negative self feedback of *Hes7* play a crucial role in controlling the somitegenesis oscillations [9]. In this section, we focus on the *Hes7* regulatory network, and see the validity of our theorems by comparing with the experimental data presented in [9]. In addition, we provide some insights obtained from the developed theorems.

Following [9], we consider the following dynamical model of the regulatory network of the Hes7 protein.

$$\begin{cases} \dot{r}(t) = -ar(t) + \frac{\beta}{1 + (p(t - \tau_p)/p_0)^2}, \\ \dot{p}(t) = -bp(t) + cr(t - \tau_r). \end{cases} \quad (35)$$

This model is equivalent to the model (1) setting $N = 1$ and $\nu = 2$. Here, the mRNA and protein degradation rates a and b are defined as

$$a = \frac{\log 2}{t_r}, b = \frac{\log 2}{t_p}, \quad (36)$$

where t_r and t_p denote mRNA and protein half-life time. We employ the parameter values for wild-type Hes7 provided in [9]: $t_p = 20$ [min], $t_r = 3$ [min], $a = 0.231$ [min^{-1}], $b = 0.0347$ [min^{-1}], $c = 4.5$ [min^{-1}], $\beta = 33$ [min^{-1}], $\tau_p = 30$ [min], $\tau_r = 7$ [min].

In [9], a point mutation in the gene was introduced, and mice expressing mutant Hes7 were generated. The protein half-life of one of the Hes7 mutants was identified as almost $t_p = 30$ minutes, which is longer than that of the wild-type Hes7, which is $t_p = 20$ minutes. As a result, the protein degradation rate of the mutant Hes7 changed to $b = 0.0231$ [min^{-1}].

Numerical simulations of the model (35) revealed that the protein of the wild-type Hes7 shows oscillations, but that of the mutant Hes7 converges to a stable equilibrium [9]. In addition, the experimental result was consistent with the numerical simulations [9].

We here present that Corollary 1 and Theorem 2 can explain these observations. First, we compute the values of the essential biological parameters, and obtain Table 1. We see from Table 1 that there exist oscillations before the mutation, because $L = 1.97 > \bar{L} = 1.85$ in Corollary 1, and equivalently $R = 21.5 > \bar{R} = 6.99$ in Theorem 2. On the other hand, the equilibrium point can be found to be locally stable after the mutation, because

Table 1: Values of the essential biological parameters in somitogenesis oscillator

Parameter	Before mutation	After mutation
N	1	1
Q	0.674	0.575
$\tilde{\tau}$	2.23	1.55
ν	2	2
R	21.5	26.4
\bar{R}	6.99	-
L	1.97	1.97
\bar{L}	1.85	2.39

$L = 1.97 < \bar{L} = 2.39$ in Corollary 1, and $\bar{L} = 2.39 > \nu = 2$ in Theorem 2. We see that these results agree with the existing experimental work addressed above.

It was concluded in [9] that short half-life time t_p of Hes7 protein is a key to the oscillations, though theoretical analysis was not performed. This hypothesis can be theoretically verified by using the presented theorems. Using Corollary 1, we can obtain the parameter region for the existence of oscillations in terms of half-life time of mRNA t_r and protein t_p in Fig. 6. We see that robust oscillations are guaranteed if the protein half-life time is shortened. For example, when mRNA half-life time is $t_r = 3$ [min], there exists oscillations for 0.1 [min] $\leq t_p \leq 22$ [min].

7 Conclusion

In this paper, we have developed analytic conditions for the existence of oscillations in cyclic gene regulatory networks with time delay. We have employed the Poincaré-Bendixson type theorem for cyclic time delay systems, and showed that a local instability of the unique equilibrium state implies the existence of oscillations. Then, we have presented the instability analysis method for linearized gene regulatory networks with time delays, which can be graphically confirmed. Using the graphical method, we have derived the analytic conditions for the existence of oscillations.

One of the distinctive features of these conditions is that they can be applied to large-scale gene regulatory networks consisting of any number of genes. Moreover, the conditions are expressed only in terms of biochemical parameters, and thus, novel biological insight is easily obtained. In particular, it has been pointed out that the normalized time delay defined in this paper serves as one of the essential physical quantities for determining the existence of oscillations. Then, the relation between the parameter and

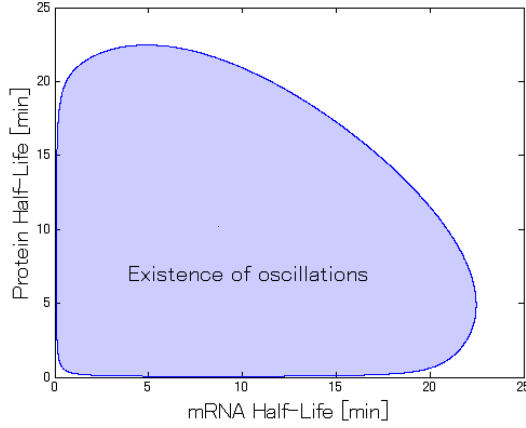


Figure 6: Parameter region for the existence of oscillations in somitogenesis oscillator. The existence of oscillations is more probable when mRNA half-life time is small, which is consistent with the hypothesis in [9].

the existence of oscillations has been revealed. Finally, we have applied the presented theorems to the existing gene regulatory networks, and observed that the results are consistent with those experimental works.

Acknowledgment

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A Transformation of the System

We here show that the gene regulatory network system defined by (1) can be obtained from (4) by the transformation (6).

We take a time derivative of $x_i(t)$ ($i = 1, 2, \dots, 2N$), and substitute (1).

$$\begin{aligned}
 \dot{x}_{2i-1}(t) &= \sigma_{2i-1} T \dot{p}_{N-i+1}(Tt - \eta_{2i-1}) \\
 &= \sigma_{2i-1} T \{ -b_{N-i+1} p_{N-i+1}(Tt - \eta_{2i-1}) + \\
 &\quad c_{N-i+1} r_{N-i+1}(Tt - \eta_{2i-1} - \tau_{r_{N-i+1}}) \} \\
 &= -b_{N-i+1} T x_{2i-1}(t) + c_{N-i+1} T \sigma_{2i-1} r_{N-i+1}(Tt - \eta_{2i-1}) \\
 &= -b_{N-i+1} T x_{2i-1}(t) + c_{N-i+1} T \rho_{2i} x_{2i}(t),
 \end{aligned} \tag{37}$$

for $i = 1, 2, \dots, N$.

$$\begin{aligned}
 \dot{x}_{2i}(t) &= \sigma_{2i} T \dot{r}_{N-i+1}(Tt - \eta_{2i}) \\
 &= \sigma_{2i} T \{ -a_{N-i+1} r_{N-i+1}(Tt - \eta_{2i}) + \\
 &\quad \beta_{N-i+1} f_{N-i+1}(p_{N-i}(Tt - \eta_{2i} - \tau_{p_{N-i}})) \} \\
 &= -a_{N-i+1} T x_{2i}(t) + \beta_{N-i+1} T \sigma_{2i} f_{N-i+1}(p_{N-i}(Tt - \eta_{2i+1})) \\
 &= -a_{N-i+1} T x_{2i}(t) + \beta_{N-i+1} T \sigma_{2i} f_{N-i+1}(\sigma_{2i+1} x_{2i+1}(t)),
 \end{aligned} \tag{38}$$

for $i = 1, 2, \dots, N - 1$, and

$$\begin{aligned}
 \dot{x}_{2N}(t) &= \sigma_{2N} T \dot{r}_1(Tt - \eta_{2N}) \\
 &= \sigma_{2N} T \{ -a_1 r_1(Tt - \eta_{2N}) + \\
 &\quad \beta_1 \sigma_{2N} f_1(p_N(Tt - \eta_{2N} - \tau_{p_N})) \} \\
 &= -a_1 T x_{2N}(t) + \beta_1 T \sigma_{2N} f_1(p_N(Tt - T)) \\
 &= -a_1 T x_{2N}(t) + \beta_1 T \sigma_{2N} f_1(x_1(t - 1)).
 \end{aligned} \tag{39}$$

We see that (37), (38) and (39) are of the form (1). Also we can verify that (37), (38) and (39) satisfy (5) as follows. It holds that

$$\frac{\partial g_{2i-1}(x_{2i-1}, x_{2i})}{\partial x_{2i}} = c_{N-i+1} T \rho_{2i}, \tag{40}$$

$$\frac{\partial g_{2i}(x_{2i}, x_{2i+1})}{\partial x_{2i+1}} = \beta_{N-i+1} T \sigma_{2i} \sigma_{2i+1} \frac{df_{N-i+1}}{dp}, \tag{41}$$

$$\frac{\partial g_{2N}(x_{2N}, x_1)}{\partial x_1} = \beta_1 T \sigma_{2N} \frac{df_1}{dp}. \tag{42}$$

It is clear from the definition that (40) is positive. We can see that (41) and (42) are also positive, because it follows that

$$\sigma_{2i}\sigma_{2i+1}\text{sgn}\left[\frac{df_{N-i+1}}{dp}\right] = \rho_{2i+1}^2 > 0 \quad \text{and} \quad \sigma_{2N}\text{sgn}\left[\frac{df_1}{dp}\right] = z^*. \quad (43)$$

Note that the sign of x_i is the same as that of σ_i , and $f_i(\cdot)$ is a monotonic function defined on positive orthant. The relation (5) can be alternatively confirmed by the following calculation.

$$\begin{aligned} \frac{\partial g_{2i-1}(x_{2i-1}, x_{2i})}{\partial x_{2i}} &= \frac{\partial r_{N-i+1}}{\partial x_{2i}} \frac{\partial g_{2i-1}}{\partial r_{N-i+1}} = \sigma_{2i} c_{N-i+1} T \sigma_{2i-1} \\ &= c_{N-i+1} T \rho_{2i}, \end{aligned} \quad (44)$$

$$\frac{\partial g_{2i}(x_{2i}, x_{2i+1})}{\partial x_{2i+1}} = \frac{\partial p_{N-i}}{\partial x_{2i+1}} \frac{\partial g_{2i}}{\partial p_{N-i}} = \sigma_{2i+1} \beta_{N-i+1} T \sigma_{2i} \frac{df_{N-i+1}}{dp}, \quad (45)$$

$$\begin{aligned} \frac{\partial g_{2N}(x_{2N}, x_1)}{\partial x_1} &= \frac{\partial p_N}{\partial x_1} \frac{\partial g_{2N}}{\partial p_N} = \sigma_1 \beta_1 T \sigma_{2N} \frac{df_1}{dp} \\ &= \beta_1 T \sigma_{2N} \frac{df_1}{dp}, \end{aligned} \quad (46)$$

where the right-hand sides coincide with those of (40), (41) and (42), respectively.

B Supplementary Description of Remark 1

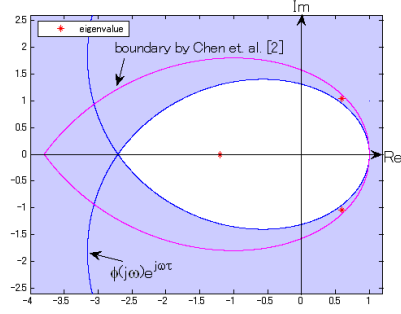
Figure 7(a) shows the comparison of Proposition 3 in this paper and Theorem 2 in [4]. The white region corresponds to Ω_+^c , which is a stability region derived from Proposition 3 in this paper.

Figure 7(b) shows the Nyquist diagram of the linearized regulatory network system, where the parameters are set as described in Remark 1, *i.e.*,

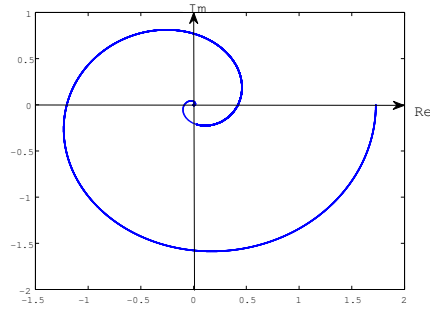
$$-\prod_{i=1}^3 \frac{c_i \beta_i}{(s + a_i)(s + b_i)} e^{-s(\tau_{r_i} + \tau_{p_i})} f'(p_{i-1}^*) = 1.2^3 \frac{e^{-3s}}{(s+1)^6}.$$

We see that the Nyquist contour encloses $-1 + j0$, which implies that the system is unstable. This contradicts Theorem 2 in [4], from which stability is concluded as seen in Fig. 7(a). Indeed, a pair of Jacobian eigenvalues of (1) is found in the right-half complex plane at $0.0212 \pm 0.3634j$ by numerical computation (see Fig. 7(c)).

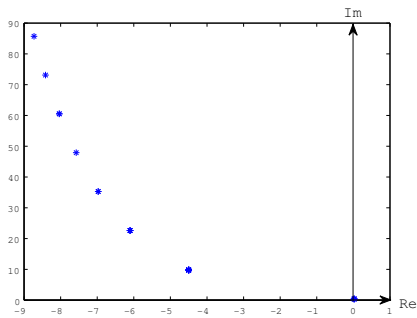
A numerical simulation of the dynamical model also verifies instability of the equilibrium point. The simulation result is shown in Fig. 8. We observe that the trajectory started around the system's equilibrium turns into oscillations. Specifically, the initial values are set as $[r_1, p_1, r_2, p_2, r_3, p_3] = [0.699, 1.224, 0.698, 1.226, 0.697, 1.225]$ in this example.



(a) Comparison of the stability regions. The white region shows Ω_+^c specified by Proposition 3 in this paper.



(b) Nyquist contour of the system. The contour encloses $-1+j0$.



(c) Roots of the characteristic equation in terms of the eigenvalue $\sqrt{1.2}e^{j\pi/6}$.

Figure 7: Proposition 3 in this paper and Theorem 2 in [4] are compared for the system described in Remark 1.

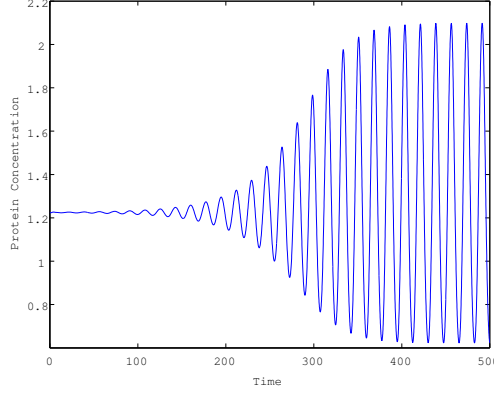


Figure 8: Time plot of the protein concentrations for the system described in Remark 1. The trajectory started in the vicinity of the equilibrium eventually exhibits the oscillations.

In what follows, we will clarify the errors of their mathematical proof. There are essentially two errors in the mathematical proof provided in [4]. First, Theorem 2.6 in [3], which is used in the proof in [4], is incorrect. Second, Theorem 2.6 in [3] was applied in a wrong way in [4].

In the remaining of this section, we use the notations defined in [3, 4] for the sake of easy comprehension and comparison. Our first claim is that Theorem 2.6 in [3] is not the necessary and sufficient, but a sufficient condition. Using the notations in [3], we see that

$$\begin{aligned}
 \lambda = -1 + be^{-\lambda\tau} &\iff \sigma e^{\sigma\tau} = be^{\tau} \\
 &\iff \begin{cases} R = \rho e^{(r-1)\tau} \\ \phi = \theta + s\tau + 2\pi j \end{cases} \\
 &\iff \begin{cases} R = \rho e^{(r-1)\tau} & \text{(a)} \\ \phi = \theta + Re^{(1-r)\tau}\tau \sin \theta & \text{(b).} \end{cases}
 \end{aligned}$$

where $\rho > 0$ and $0 \leq \theta < 2\pi$ ³. Note that the equations (a) and (b) are the same as (2.7a) and (2.8) in [3]. It was concluded that all roots λ of the above equation have negative real parts if and only if $(R, \phi) \in \{(R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \text{(a) and (b) only if } r < 1\}$. Then, the stability region was considered by specifying (R, ϕ) that belongs to the above set. It follows that

$$\{(R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \text{(a) and (b) only if } r < 1\} \quad (47)$$

$$\supsetneq \{(R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \text{(b) only if } r < 1\} \quad (48)$$

$$= \overline{\{(R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \text{(b) for some } r \geq 1\}}, \quad (49)$$

³Equation (2.4) in [3] is typo. It should be corrected as $\lambda = -1 + be^{-\lambda\tau}$.

where $\overline{\{\cdot\}}$ denotes a complementary set. Then, the set (49) was specified in Theorem 2.6 in [3] as

$$\begin{aligned} & \overline{\{(R, \phi) \in [0, \infty) \times [0, 2\pi) \mid (b) \text{ for some } r \geq 1\}}, \\ &= \overline{\left\{ (R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \phi \leq \frac{\pi}{2} + R\tau \text{ or } \phi \geq \frac{3\pi}{2} - R\tau \right\}} \\ &= \left\{ (R, \phi) \in [0, \infty) \times [0, 2\pi) \mid \phi > \frac{\pi}{2} + R\tau \text{ and } \phi < \frac{3\pi}{2} - R\tau \right\}. \end{aligned} \quad (50)$$

In [3], however, (48) was not derived as a subset but as an equivalent set of (47). Therefore, it was concluded that (50) provides the stability region where all the eigenvalues of the system are located, *if and only if* the system is asymptotically stable. This conclusion is, however, incorrect, because (48) is actually a subset of (47).

Instead, we see that (50) is the region where all the eigenvalues of the system are located, *if* the system is asymptotically stable. Therefore, we claim that Theorem 2.6 in [3] provides only a sufficient condition for stability.

The other error of Theorem 2 in [4] stems from the mis-application of Theorem 2.6 in [3]. The boundary of the stability region provided in Theorem 2.6 in [3] is the Archimedean spiral starting from the origin. Applying Theorem 2.6 in [3] to the equation (11) in [4], we see that the boundary of the stability region is given by

$$R = \begin{cases} \frac{2\theta - \pi}{k\tau} & \text{for } \frac{\pi}{2} < \theta < \pi \\ \frac{3\pi - 2\theta}{k\tau} & \text{for } \pi < \theta < \frac{3\pi}{2}, \end{cases} \quad (51)$$

where the constants k and τ are defined as in [4]. The tuple (R, θ) defines the distance and the angle of the boundary measured from the origin. Consequently, the arc drawn by (51) becomes the well-known Archimedean spiral.

In Theorem 2 of [4], however, the left-hand side of (51), R , was shifted by one, and the equation of the boundary was given by

$$R - 1 = \begin{cases} \frac{2\theta - \pi}{k\tau} & \text{for } \frac{\pi}{2} < \theta < \pi \\ \frac{3\pi - 2\theta}{k\tau} & \text{for } \pi < \theta < \frac{3\pi}{2}. \end{cases} \quad (52)$$

Then, R and θ are measured from the origin and $1 + j0$, respectively (see also Fig. 2 in [4]). It is clear that the boundary obtained in this way does not coincide with the one in Theorem 2.6 in [3].

C Proof of Corollary 1

We observe that the left-hand side of (23) is the monotonically increasing function of L , and the right-hand side is the monotonically decreasing function of L . Moreover, we can see that the inequality (23) is not satisfied when $L \leq 1$, but it is satisfied when $L > W(N, Q)$. Therefore, we have a critical value \bar{L} at which the left-hand side and the right-hand side of (23) take the same value, and the inequality (23) is satisfied if and only if $L > \bar{L}$. It is clear from the above argument that \bar{L} is given as the unique solution of (26), and \bar{L} satisfies $1 < \bar{L} \leq W(N, Q)$. \square