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Existence Criteria of Periodic Oscillations in Cyclic Gene Regulatory Networks

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

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Abstract

In this paper, we derive graphical and analytic criteria for the existence of periodic oscillations in large-scale cyclic gene regulatory networks, and present quantitative biological insight based on the analytic result. Based on the Poincaré-Bendixson theorem for cyclic systems, it is first shown that local instability of an equilibrium point implies the existence of periodic oscillations. Then, we prove that local instability of the heterogeneous gene regulatory network system, where dynamics of gene expression is considerably different between genes, is satisfied, if the homogeneous system is locally unstable. From this observation, the simple graphical and its equivalent analytic criteria for the existence of periodic oscillations are derived by local instability analysis of homogeneous cyclic gene regulatory networks. These criteria have the remarkable feature that they are easily applied to a large-scale cyclic gene regulatory network systems consisting of arbitrary number of genes, and thus, dynamical properties of such large-scale systems can be systematically obtained. The latter part of this paper is devoted to the rigorous investigation of the nonlinear dynamical properties. Specifically, we examine the relation between an equilibrium state and biochemical parameters, and present the analytic criterion which takes the dependence of the equilibrium state on biochemical parameters into account. Since this rigorous analytic criterion depends only on the given biochemical parameters, one can easily obtain the relation between biochemical parameters and the existence of periodic oscillations. In particular, we propose the decisive physical quantities for the existence of periodic oscillations, and reveal the quantitative relation between such physical quantities and the dynamical behaviors. Finally,

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transportation time delay is introduced into the dynamical model, and the effects of such delay are briefly discussed.

Keywords: Gene regulatory network; Systems biology; Large-scale systems; Periodic oscillation; Nonlinear systems

1 Introduction

Gene regulatory network refers to a chemical interaction network between genes and proteins in a cell. With the rapid progress of molecular biology, it has been revealed that these mutual chemical interactions produce complex dynamical behaviors of protein levels in a cell, and that such dynamical behaviors play a key role to maintain fundamental biological functions of living organisms such as circadian rhythms (see Leloup and Goldbeter (2008) and references therein). Recently, *Systems biology* and *Synthetic biology*, new interdisciplinary research field between biology and engineering, have emerged to unravel quantitative properties of the gene regulatory networks. In these lines of research, theoretical analysis based on mathematical models is indispensable to systematically understand the relation between the biochemical parameters and the dynamical behaviors of protein concentrations. In particular, a unified analysis scheme for examining oscillations in *large-scale* gene regulatory networks is now strongly required.

Existing mathematical models of gene regulatory networks can be mainly classified into two types: stochastic and deterministic modeling. In stochastic modeling (Gillespie, 1992), the randomness of molecular interactions in biological networks is explicitly considered, and an efficient algorithm (Gillespie, 1976) has been developed to examine the stochastic nature of molecular levels in biological networks. On the other hand, deterministic differential equation models have been widely used to capture relatively macroscopic dynamical behaviors, and the detailed quantitative relation between biochemical parameters and dynamical behaviors has been investigated in many previous works (see for instance, Samad et al. (2005); Ugander et al. (2007); Vecchio (2007); Wang et al. (2004); Chesi and Hung (2008)).

One of the pioneering theoretical analyses of gene regulatory network was presented by Goodwin (1965), 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 (Stephanopoulos et al., 1998; Kholodenko, 2000), and it is recently considered that cyclic structure plays a key role to produce the various dynamical behaviors of protein levels. In fact, the artificially constructed biological oscillator, Repressilator (Elowitz and Leibler, 2000), was performed with a simple cyclic interaction of repressors in *Escherichia coli*. Also, theoretical analysis has shown that the cyclic structure is simple but important to maintain the oscillations of protein concentrations (Trané and Jacobsen, 2008).

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.

Samad et al. (2005) have presented the analytic criteria for the existence of oscillations in cyclic gene regulatory networks based on the result in Hastings et al. (1977). A key feature of their result is that the criteria are explicitly expressed in terms of biochemical parameters, and thus, one can easily observe the relation between the parameters and the periodic oscillations of protein concentrations. These criteria were obtained by directly calculating the eigenvalues of the Jacobian matrix. However, this approach becomes considerably difficult as the number of genes, equivalently the size of the Jacobian matrix, gets large. Thus, it is desirable to develop a novel analysis scheme that is independent on the scale of the gene regulatory network. In particular, it is important to note that the equilibrium state, at which the system is linearized, usually changes depending on the biochemical parameters due to the inherent nonlinearity of gene regulatory networks. Therefore, the dependence of the equilibrium state on biochemical parameters should be explicitly treated in analyzing the existence of periodic oscillations.

The objective of this paper is to derive existence criteria of periodic oscillations of protein concentrations in *large-scale* cyclic gene regulatory networks, and present novel biological insights. We perform both graphical and analytic criteria which can be applied to cyclic gene regulatory networks consisting of any number of genes. In particular, the developed analytic criterion explicitly takes the dependence of the equilibrium on biochemical parameters into account despite it is derived based on local instability analysis. Thus, significant biological insights are obtained from the analytic result. Specifically, we propose the novel physical quantities that are essential for determining the existence of periodic oscillations, and reveal the quantitative relation between the existence of periodic oscillations and the physical quantities.

To this end, we first show that the existence condition of periodic oscillations can be reduced to local instability condition of the equilibrium state based on the Ponicaré-Bendixson theorem for cyclic systems (Mallet-Paret and Smith, 1990), and then, prove that the existence of periodic oscillations in *heterogeneous* gene regulatory networks, where dynamics of gene expression is considerably different between genes, can be guaranteed if it is guaranteed in gene regulatory networks with *homogeneous* gene expression dynamics. Then, local instability analysis leads the graphical criterion. Though this graphical criterion has been originally presented for the homogeneous gene regulatory networks (Hori et al. (2009a)), it now covers the heterogeneous gene regulatory networks as well. Then, we reveal the important relation between the equilibrium state and biochemical parameters. This leads to the analytic criterion for the existence of periodic oscillations, and biological insights obtained from the criterion are performed with illus-

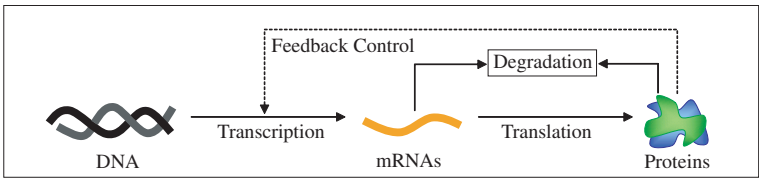


Figure 1: Mechanism of protein synthesis in gene regulatory networks

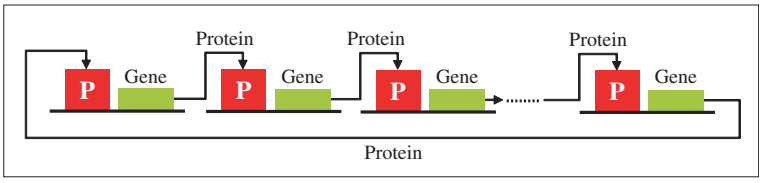


Figure 2: Gene regulatory networks with cyclic structure

trative numerical simulations.

Finally, transportation time delay, which is sometimes unignorable especially for eukaryotic cells (Chen and Aihara, 2002), is introduced into the dynamical model, and the effects of such time delay are briefly discussed by extending our analysis scheme developed for the non-delay case. The developed scheme reduces the tough analysis of nonlinear time delay system to a simple graphical criterion, and thus it would be useful for extensive future research.

2 Model description of gene regulatory networks

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 is first transcribed into messenger RNA(mRNA), and then a mRNA is translated into one or multiple copies of corresponding proteins. Furthermore, some proteins, called transcription factors, are known to activate or repress the transcription of other genes. Such chemical interactions between transcription factors and genes can be described by gene regulatory networks (see Fig. 1).

The gene regulatory networks where each protein activates or represses another transcription in a cyclic way as in Fig. 2, are called *cyclic* gene regulatory networks. The dynamics of mRNA and protein concentrations in the cyclic gene regulatory networks with N genes 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 $r_i \in \mathbb{R}_+ (:= \{x \in \mathbb{R} \mid x \geq 0\})$ and $p_i \in \mathbb{R}_+$ denote the normalized

concentrations of the i-th mRNA and its corresponding protein synthesized in the i-th gene, respectively (Elowitz and Leibler, 2000; Samad et al., 2005) [1]. 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 of 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 $f_i(\infty) = 0$ (monotonically decreasing), whereas for activation as $f_i(0) = 0$ and $f_i(\infty) = 1$ (monotonically increasing). In practical applications, the following Hill function is often introduced to describe biochemical characterization:

$$f_i(p_{i-1}) = \begin{cases} \dfrac{1}{1+p_{i-1}^{\nu}} (=: F_R(p_{i-1})) & \text{(for repression)} \\ \dfrac{p_{i-1}^{\nu}}{1+p_{i-1}^{\nu}} (=: F_A(p_{i-1})) & \text{(for activation)} \end{cases} \tag{2}$$

where $\nu(\geq 1) \in \mathbb{R}_+$ is a Hill coefficient, which represents a degree of cooperative binding, and determines the nonlinearity of the system (Alon, 2006).

Let δ be defined as

$$\delta := \left(\frac{df_1}{dp}\right) \cdot \left(\frac{df_2}{dp}\right) \cdots \left(\frac{df_N}{dp}\right). \tag{3}$$

The system belongs to a class of cooperative systems (Smith, 1995) when $\delta > 0$, and dynamical properties of such systems have been investigated in many previous works (see Smith (1995) and references therein). Smith (1987) and Samad et al. (2005) have shown that almost all solutions of (1) asymptotically converge to one of equilibria when $\delta > 0$. On the other hand, the protein concentrations exhibit oscillatory behaviors as well as convergence when $\delta < 0$, and the detailed study is required to clarify the relation between the parameters and the solution trajectories. Therefore, we focus on the cyclic gene regulatory networks that satisfy the following assumption in this paper.

Assumption 1. *For given $f_i(\cdot)$ $(i = 1, 2, \cdots, N)$, $\delta < 0$ is satisfied.*

This assumption means that a given gene regulatory network has an odd number of repressive interactions between genes.

3 Characterization of omega-limit set

In this section, we examine the omega-limit set of cyclic gene regulatory networks modeled by the nonlinear differential equations of (1), and show

[1] r_i and p_i are normalized by activation/repression coefficient in the Hill function, and dimensionless quantities.

that the omega-limit set of (1), in fact, consists of either an equilibrium state or limit cycles.

Although the dynamical behavior of high dimensional nonlinear system can be very complicated, Mallet-Paret and Smith (1990) gave a key result which characterizes the omega-limit set of cyclic feedback systems (see Theorem 4.1 in Mallet-Paret and Smith (1990)).

Proposition 1 (Mallet-Paret and Smith (1990))**.** *For the system (1), if all of the following conditions (a),(b) and (c) hold, then the omega-limit set consists of either (i) an equilibrium state* $\boldsymbol{q}^* := [r_1^*, p_1^*, r_2^*, p_2^*, \cdots, r_N^*, p_N^*] \in \mathbb{R}^{2N}$*, (ii) a non-constant periodic orbit, or (iii)* $\boldsymbol{q}^*$ *together with a collection of orbits homoclinic to* $\boldsymbol{q}^*$*:*

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

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

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

Furthermore, if the following condition also holds then either (i) or (ii) occurs:

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

where J *is the Jacobian matrix of (1) evaluated at* $\boldsymbol{q}^*$*.*

This proposition restricts a class of omega-limit set of the system, and rules out chaotic behavior of the solution as in the Poincaré-Bendixson theorem for two dimensional systems (Khalil, 2001). Indeed, it is proven that all the solution trajectories of (1) can be embedded into a two dimensional subspace by a planner projection.

In the following, we show that (1) satisfies the above conditions (a), (b), (c) and (d). Regarding (a) and (b), the following result is presented in Samad et al. (2005).

Lemma 1 (Samad et al. (2005))**.** *For the system (1),* $\mathbb{R}_+^{2N}$ *is a positively invariant set. In particular, all orbits starting from* $\boldsymbol{q}(0) \in \mathbb{R}_+^{2N}$ *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 $\boldsymbol{q}(\cdot)$ 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). It follows from the definition of the equilibria that

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

where $R_i^2 := (c_i \beta_i)/(a_i b_i)$ $(i = 1, 2, \cdots, N)$ and the notation $\circ$ denotes the composition of function. Then, the monotonicity of the both sides of (4) leads the following lemma (See Appendix A for proof).

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

Note that the equation (4) is generally highly complicated, but the equilibrium point can be effectively obtained by using a bisectional search algorithm due to the monotonicity.

Condition (d) can be verified by simple calculation (see Appendix B and Remark 4.1 in Mallet-Paret and Smith (1990)).

Lemma 3. *Denote the Jacobian of (1) evaluated at* $\boldsymbol{q}^*$ *by* J*. Then,* $\det(-J) > 0$ *holds.*

Thus, it is concluded that the conditions (a), (b), (c) and (d) in Proposition 1 hold for the system (1). This implies that the protein concentrations $p_i(t)$ $(i = 1, 2, \cdots, N)$ in (1) exhibits either (i) convergence to a unique equilibrium state or (ii) periodic oscillations, and homoclinic and chaotic behaviors are ruled out. From this observation, we obtain the following key proposition.

Proposition 2. *Consider the cyclic gene regulatory network systems modeled by (1). Then, the system has periodic oscillations if the unique equilibrium point of the system is locally unstable.*

Proof. If the linearized system is unstable, there exists a set of initial values in the neighborhood of the equilibrium state such that the trajectory goes away from the equilibrium state. Since homoclinic orbit is ruled out, the trajectory starting from the unstable manifold eventually enters into a non-constant periodic orbit. □

This proposition enables us to check the existence of periodic oscillations by local stability analysis of the unique equilibrium point, though it is generally difficult to show the existence of periodic oscillations in nonlinear systems. Thus, local instability conditions for the cyclic gene regulatory networks systems are considered in the next section.

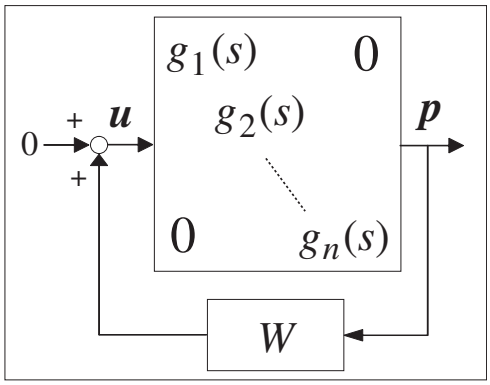


Figure 3: Block diagram of the linearized gene regulatory network system: heterogeneous gene regulatory network $\mathcal{G}(s)$

4 Local instability analysis of heterogeneous gene regulatory networks

As shown in Proposition 2, there exists a set of initial values so that protein concentrations do not converge to the equilibrium state and eventually enter into a non-constant periodic orbit if the unique equilibrium state is locally unstable. Though conventional local stability analysis methods such as Routh and Hurwitz algorithms may be available to check the local stability of the equilibrium state, these approaches have a potential drawback 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 the reasonable analysis method of which the computation burden does not highly depend on the number of genes, which is one of our research objectives.

Let $\boldsymbol{q}^* := [r_1^*, p_1^*, r_2^*, p_2^*, \cdots, r_N^*, p_N^*] \in \mathbb{R}^{2N}$ denote an equilibrium point of (1). Then, the state space representation of the linearized system of (1) at $\boldsymbol{q}^*$ can be expressed as, for $i = 1, 2, \cdots, N$,

$$\begin{bmatrix} \dot{r}_i \\ \dot{p}_i \end{bmatrix} = \begin{bmatrix} -a_i & 0 \\ c_i & -b_i \end{bmatrix} \begin{bmatrix} r_i \\ p_i \end{bmatrix} + \begin{bmatrix} \beta_i \\ 0 \end{bmatrix} u_i, \quad u_i := \zeta_i p_{i-1}, \tag{5}$$

where

$$\zeta_i := f_i'(p_{i-1}^*). \tag{6}$$

Note that $\zeta_i > 0$ when $f_i(\cdot) = F_A(\cdot)$, and $\zeta_i < 0$ when $f_i(\cdot) = F_R(\cdot)$, and Assumption 1 coincides with the inequality $\prod_{i=1}^N \zeta_i < 0$. Then, mRNA and protein concentrations of the i-th gene, r_i and p_i in (5), can be interpreted from a control theoretic viewpoint as the internal states of the system, and the transfer function of the i-th gene, $g_i(s)$, from the input u_i to the protein

concentration p_i is obtained as

$$g_i(s) := \frac{c_i\beta_i}{(s+a_i)(s+b_i)}. \tag{7}$$

Consequently, the overall dynamics of the cyclic gene regulatory network system $\mathcal{G}(s)$ is expressed as shown in Fig. 3, where $\boldsymbol{u} = W\boldsymbol{p}$ with $\boldsymbol{u} := [u_1, u_2 \cdots, u_N]^T \in \mathbb{R}^N$ and $W := \mathrm{cyc}(\zeta_1, \zeta_2, \cdots, \zeta_N) \in \mathbb{R}^{N\times N}$, where the notation $\mathrm{cyc}(\cdot)$ stands for the constant matrix defined as

$$\mathrm{cyc}(x_1, x_2, x_3, \cdots, x_N) := \begin{bmatrix} 0 & 0 & 0 & \cdots & x_1 \\ x_2 & 0 & 0 & \cdots & 0 \\ 0 & x_3 & 0 & \cdots & 0 \\ \vdots & \vdots & \ddots & \ddots & \vdots \\ 0 & 0 & \cdots & x_N & 0 \end{bmatrix}. \tag{8}$$

In gene regulatory networks, however, dynamical uncertainty of gene expression is one of important features (Stelling et al., 2007), and such uncertainty should be explicitly taken into account. Thus, parametric perturbations to $\mathcal{G}(s)$ is considered in the following analysis, though other types of perturbations may be treated within the framework of the authors' previous work (Hori et al., 2009b).

Suppose only the upper and lower bounds of each parameter value are given by $\underline{a} \le a_i \le \overline{a}$, $\underline{b} \le b_i \le \overline{b}$, $\underline{c} \le c_i \le \overline{c}$ and $\underline{\beta} \le \beta_i \le \overline{\beta}$ for $i = 1, 2, \cdots, N$. Both perturbation of the Hill coefficient ν in (2) and the dependence of the equilibrium point p^*_{i-1} in ζ_i on the perturbed parameters are simultaneously treated by perturbation of ζ_i, *i.e.*, $\underline{\zeta}_i \le |\zeta_i| \le \overline{\zeta}_i$ for given positive bounds, $\underline{\zeta}_i$ and $\overline{\zeta}_i$. The existence condition of periodic oscillations is considered by specifying the worst case parameter values of $(a_i, b_i, c_i, \beta_i, \zeta_i)$ that guarantees the instability of $\mathcal{G}(s)$.

Define $\gamma(s) := (s+b_1)\prod_{i=2}^N 1/g_i(s)$ and $v := |\prod_{i=1}^N \zeta_i|$. Then, the characteristic polynomial of $\mathcal{G}(s)$ is written as

$$\prod_{i=1}^N \frac{1}{g_i(s)} + \prod_{i=1}^N |\zeta_i| = \frac{1}{c_1\beta_1}(s+a_1)\gamma(s) + v = 0. \tag{9}$$

In the sequel, we examine the worst case parameter values of (a_i, b_i, c_i, β_i) for instability of $\mathcal{G}(s)$. It follows from the gain and phase conditions of (9) that for given (a_i, b_i, c_i, β_i), the critical gain v^* for instability can be expressed as

$$v^* = \frac{1}{c_1\beta_1}\sqrt{a_1^2+\omega^2}|\gamma(j\omega)| \tag{10}$$

$$\text{such that } \angle(a_1 + j\omega) = \pi - \angle\gamma(j\omega), \tag{11}$$

where the critical gain refers to the value of v that satisfies (9). We can easily see that $\mathcal{G}(s)$ tends to be unstable as v gets large, because v can be regarded as the feedback gain of $\mathcal{G}(s)$. It means that the parameter values of (a_i, b_i, c_i, β_i) becomes the worst case for instability of $\mathcal{G}(s)$ when v^* achieves a minimum. The following lemma gives an important relationship between the parameters (a_i, b_i, c_i, β_i) and the critical gain v^*, and greatly simplifies our analysis.

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 (10) and (11), 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 $a_\nu > a_\rho$. It is obvious that

$$\angle(a_\nu + j\omega_\rho) < \angle(a_\rho + j\omega_\rho) = \pi - \gamma(j\omega_\rho). \tag{12}$$

Moreover, it follows from (12) that there exists $\omega_\nu (> \omega_\rho)$ such that $\angle(a_\nu + j\omega_\nu) = \pi - \gamma(j\omega_\nu)$ because $\angle\gamma(j\omega)$ is a monotonically increasing function with respect to ω. Then, the critical gain v_ν^* for instability of $\mathcal{G}(s)$ with (a_ν, ω_ν) is obtained as

$$v_\nu^* = \frac{1}{c_1\beta_1}\sqrt{a_\nu^2 + \omega_\nu^2}|\gamma(j\omega_\nu)|. \tag{13}$$

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. Following the similar arguments, the above condition is also proven for $a_2, a_3, \cdots, a_N$ and $b_1, b_2, \cdots, b_N$.

On the other hand, since the phase condition (11) is not affected by the change of c_i and β_i $(i = 1, 2, \cdots, N)$, it immediately follows from (10) that the critical gain monotonically decreases with respect to c_i and β_i. □

This lemma implies that the critical gain v^* achieves a minimum 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 is obtained.

Proposition 3. *Consider the cyclic gene regulatory networks modeled by (1) and its linearized system $\mathcal{G}(s)$. Suppose $\underline{a} \le a_i \le \overline{a}$, $\underline{b} \le b_i \le \overline{b}$, $\underline{c} \le c_i \le \overline{c}$, $\underline{\beta} \le \beta_i \le \overline{\beta}$ and $\underline{\zeta}_i \le |\zeta_i| \le \overline{\zeta}_i$ $(i = 1, 2, \cdots, N)$ are satisfied for given*

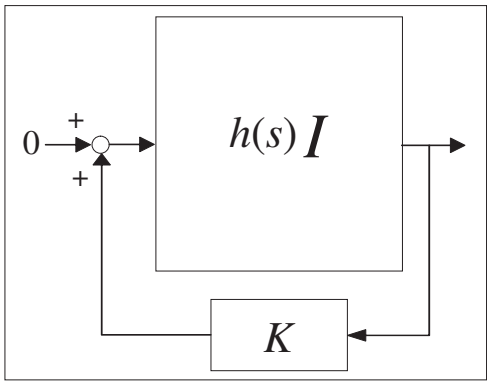


Figure 4: Block diagram of the linearized gene regulatory network system: homogeneous gene regulatory network $\mathcal{H}(s)$.

$\underline{a}$, $\overline{a}$, $\underline{b}$, $\overline{b}$, $\underline{c}$, $\overline{c}$, $\underline{\beta}$, $\overline{\beta}$, $\underline{\zeta}_i$ and $\overline{\zeta}_i$. Then, there exist periodic oscillations of protein concentrations p_i ($i = 1, 2, \cdots, N$) if the linear system $\mathcal{G}(s)$ with $a_i = \overline{a}, b_i = \overline{b}, c_i = \underline{c}, \beta_i = \underline{\beta}$ and $\zeta_i = z_i\underline{\zeta}_i$ is unstable, where $z_i = +1$ if $f_i(\cdot) = F_A(\cdot)$ and $z_i = -1$ if $f_i(\cdot) = F_R(\cdot)$ ($i = 1, 2, \cdots, N$).

Proof. It follows from the above arguments that $\mathcal{G}(s)$ is unstable for any $(a_i, b_i, c_i, \beta_i, \zeta_i)$ $(i = 1, 2, \cdots, N)$ satisfying the given lower and upper bounds if and only if $\mathcal{G}(s)$ with $a_i = \overline{a}$, $b_i = \overline{b}$, $c_i = \underline{c}$, $\beta_i = \underline{\beta}$ and $\zeta_i = z_i\underline{\zeta_i}$ $(i = 1, 2, \cdots, N)$ is unstable. The conclusion immediately follows since the gene regulatory network system modeled by (1) has periodic oscillations if $\mathcal{G}(s)$ is unstable (see Proposition 2). □

This proposition means that the existence of periodic oscillations can be confirmed by checking the local stability of the gene regulatory network system with $a_i = \overline{a}, b_i = \overline{b}, c_i = \underline{c}, \beta_i = \underline{\beta}, \zeta_i = z_i\underline{\zeta}_i$ $(i = 1, 2, \cdots, N)$. Our analysis is now greatly simplified because we only need to consider the *homogeneous* cyclic gene regulatory networks where the dynamics of each gene expression is identical between genes. Therefore, we hereafter focus on the *homogeneous* gene regulatory network, and derive the existence condition of periodic oscillations for the heterogeneous gene regulatory networks modeled by (1).

5 Criteria for the existence of periodic oscillations

In this section, graphical and analytic criteria for the existence of periodic oscillations of protein concentrations are presented for the homogeneous cyclic gene regulatory networks. For the sake of notation simplicity, we denote $a := \overline{a}, b := \overline{b}, c := \underline{c}, \beta := \underline{\beta}$ and $\xi_i := z_i\underline{\zeta}_i$ $(i = 1, 2, \cdots, N)$ where z_i is defined as mentioned in Proposition 3.

5.1 Graphical criterion

Consider the linear system $\mathcal{G}(s)$ depicted in Fig. 3 where $a_i = a, b_i = b, c_i = c, \beta_i = \beta$ and $\zeta_i = \xi$ for $i = 1, 2, \cdots, N$. Since the feedback gain matrix W has a special structure, the gain $c_i\beta_i/a_ib_i$ of $g_i(s)$ can be merged into the corresponding feedback gains in W, and the original feedback system of Fig. 3 can be transformed into the system $\mathcal{H}(s)$ shown in Fig. 4 where

$$h(s) := \frac{1}{(T_r s + 1)(T_p s + 1)} \tag{14}$$

with $T_r := 1/a$, $T_p = 1/b$, and

$$K := R^2 \cdot \mathrm{cyc}(\xi_1, \xi_2, \cdots, \xi_N) \ \in \mathbb{R}^{N\times N} \tag{15}$$

with $\mathrm{cyc}(\cdot)$ defined by (8). The dimensionless quantity R is defined as

$$R := \frac{\sqrt{c\beta}}{\sqrt{ab}}. \tag{16}$$

Note that R is the ratio between the geometric means of degradation and production rates, and is one of the important quantities that determine the existence of periodic oscillations, as will be shown in later. Then, the overall transfer function of the linearized homogeneous gene regulatory network system $\mathcal{H}(s)$ is obtained as

$$\mathcal{H}(s) := (\phi(s)I - K)^{-1}, \ \phi(s) := \frac{1}{h(s)}. \tag{17}$$

Therefore, instability of the linear system $\mathcal{H}(s)$ implies the existence of periodic oscillations in cyclic gene regulatory networks (see Proposition 2).

Here we point out that $\mathcal{H}(s)$ is regarded as a system with a generalized frequency variable $\phi(s)$ (Hara et al., 2009; Tanaka et al., 2009). Thus, the stability analysis scheme presented in Hara et al. (2009) leads the following graphical criterion for the existence of periodic oscillations of protein concentrations.

Theorem 1. *Consider the heterogeneous cyclic gene regulatory network systems modeled by (1), and the linear system $\mathcal{H}(s)$ in (17). Then, the system has periodic oscillations of protein concentrations p_i ($i = 1, 2, \cdots, N$) if at least one of the eigenvalues of K in (15) lies inside the domain Ω_+ defined by*

$$\Omega_+ := \phi(\mathbb{C}_+) = \{\lambda \in \mathbb{C} \mid \exists s \in \mathbb{C}_+ \text{ s.t. } \phi(s) = \lambda\}. \tag{18}$$

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

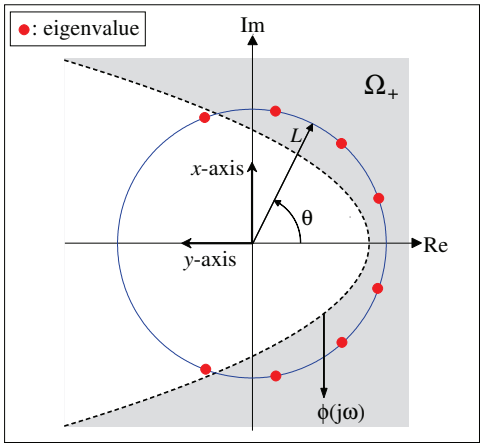


Figure 5: Domain Ω_+ and eigenvalue distribution of K.

between genes, and the domain Ω_+ which is determined by the homogeneous gene dynamics $h(s)$. In particular, it is worth noting that stability analysis of the *large-scale* gene regulatory networks can be done with the eigenvalues of the relatively small matrix K and $\phi(s)$ in (17). Specifically, the eigenvalues of K are computed as, for $k = 1, 2, \cdots, N$,

$$\lambda_k = Le^{j(2k-1)\pi/N}, \quad L := R^2 \left| \prod_{\ell=1}^{N} \xi_\ell \right|^{\frac{1}{N}}, \tag{19}$$

which implies that all the eigenvalues of K are located on a circle with a center at the origin and a radius of L (see Fig. 5). Therefore, the existence of periodic oscillations can be easily confirmed from the eigenvalues on the circle and the curve defined by $\phi(j\omega)$.

We remark that the condition presented in Theorem 1 is the necessary and sufficient condition for local instability of $\mathcal{H}(s)$. Thus, we can also conclude that there exists a set of initial values for which the protein concentrations in the *homogeneous* cyclic gene regulatory network converge to the equilibrium if and only if Theorem 1 does not hold.

5.2 Analytic criterion

In this section, we investigate detailed properties of the graphical criterion, and derive an equivalent analytic criterion for the existence of periodic oscillations.

First, we consider an analytic expression of the boundary $\phi(j\omega)$ of the domain Ω_+. Let the x-y coordinate be defined as illustrated in Fig. 5. Then,

the domain Ω_+ can be expressed in terms of $x := \mathrm{Im}[s]$ and $y := -\mathrm{Re}[s]$ as

$$\Omega_+ := \left\{ -y + jx \in \mathbb{C} \mid y < \frac{1}{4}Q^2x^2 - 1 \right\}, \tag{20}$$

where

$$Q := \frac{\sqrt{T_rT_p}}{(T_r + T_p)/2} \left(= \frac{\sqrt{ab}}{(a+b)/2} \right). \tag{21}$$

Obviously, the boundary of the region Ω_+ becomes a parabolic curve characterized by Q. The dimensionless quantity Q is the ratio between the arithmetic and geometric means of the degradation time constants of mRNA and protein, T_r and T_p, and is one of the essential physical quantities for determining the existence of periodic oscillations as will be shown in later. It should be noted that it follows from the definition that $0 < Q \leq 1$ and the equality holds if and only if $T_r = T_p$.

Based on the above characterization of Ω_+ and the eigenvalue distribution of K in (19), an analytic criterion for the existence of periodic oscillations in large-scale cyclic gene regulatory networks is obtained.

Theorem 2. *Consider the heterogeneous cyclic gene regulatory network systems modeled by (1) and the linear system $\mathcal{H}(s)$ in (17). Then, the system has periodic oscillations of protein concentrations p_i ($i = 1, 2, \cdots, N$), if*

$$L > W(N, Q), \tag{22}$$

where

$$W(N,Q) := \frac{2\left(-\cos(\frac{\pi}{N}) + \sqrt{\cos^2(\frac{\pi}{N}) + Q^2\sin^2(\frac{\pi}{N})}\right)}{Q^2\sin^2(\frac{\pi}{N})}. \tag{23}$$

Proof. Consider $\phi(s)$ defined by (17). We first claim that $|\phi(j\omega)|$ monotonically increases as $|\omega|$ increases, and $\arg(\phi(j\omega))$ monotonically increases as ω increases. The monotonicity of $\arg(\phi(j\omega))$ is clear from (18)(see Fig. 5). The monotonicity of $|\phi(j\omega)|$ is also verified from (18), *i.e.,*

$$|\phi(j\omega)| = \sqrt{x^2 + y^2} = \sqrt{x^2 + \left(\frac{1}{4}Q^2x^2 - 1\right)^2} \tag{24}$$

monotinically increases with respect to x.

Recall that eigenvalues of K are given by (19), which means that all the eigenvalues are located on a circle with a center at the origin and a radius of L. Then, it is follows that λ_1 and λ_N, which are the eigenvalues with the largest real part, always reach the boundary of Ω_+, *i.e.,* $\phi(j\omega)$, first,

because of the monotonicity claimed above. Therefore, λ_1 and λ_N lie inside Ω_+ if and only if L is greater that $|\lambda_1|(= |\lambda_N|)$.

Let $x = \mathrm{Im}[s]$ and $y = -\mathrm{Re}[s]$ be rewritten as $x = d\sin\theta$, and $y = -d\cos\theta$, where $d \in \mathbb{R}$ is the distance between the origin and the boundary, and θ is taken positive in counter-clockwise direction as illustrated in Fig.5. Then, we obtained

$$d = \frac{2\left(-\cos\theta + \sqrt{\cos^2\theta + Q^2\sin^2\theta}\right)}{Q^2\sin^2\theta} \tag{25}$$

by substituting the above x and y into $y = (Q^2x^2)/4 - 1$, and solving the equation. Therefore, the conclusion follows by writing the condition follows by evaluating the distance d at $\theta = \pi/N$ where λ_1 is located. □

The above condition (22) is equivalent to the graphical one in Theorem 1. In particular, the left-hand side of (22) stands for the radius of the circle where all the eigenvalues are located, while 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., $\theta = \pi/N$ in Fig. 5). By using this analytic criterion we can easily check the existence of periodic oscillations in heterogeneous cyclic gene regulatory networks with a large number of genes.

Finally, we briefly remark on the relation between our results and the conventional one. A condition for instability of $\mathcal{H}(s)$ has been obtained in Thron (1991) in the context of biochemical analysis. It, however, gives a necessary condition for instability, while Theorem 1 and Theorem 2 are necessary and sufficient. Thus, the criteria obtained in this paper are more strict than the previous one.

5.3 Numerical simulations

The synthetic biological oscillator, Repressilator, was performed in *Escherichia coli* with cyclic gene regulatory networks consisting of three genes (Elowitz and Leibler, 2000). In this example, we consider the same negative feedback structure as Repressilator except that the number of genes is increased to see that our criteria can effectively work for large-scale cyclic gene regulatory networks. We remark that the leakiness term modeled in Elowitz and Leibler (2000) is omitted here, but the analysis can be done in a similar fashion even if there is the one.

Example 1. Consider the cyclic gene regulatory network composed of $N = 7$ genes with the dynamics in (1), where all the interactions between genes are repressive, *i.e.,* $f_i(\cdot) = F_R(\cdot)$ in (2). Suppose the exact values of the parameters are not known due to the inherent uncertainty of the dynamics, but the ranges are specified as $0.5 \le a_i \le 1.5, 1.0 \le b_i \le 2.5, 8.0 \le c_i \le 10.0$ and $8.5 \le \beta_i \le 10.0$ for $i = 1, 2, \cdots, N$. Moreover, suppose $\xi_1 = -0.10, \xi_2 =$

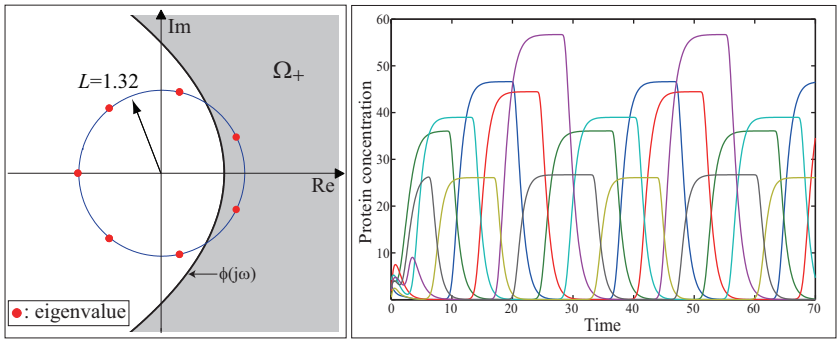


Figure 6: (Right) The domain Ω_+ and the eigenvalues of K: Two eigenvalues belong to Ω_+. (Left) Time plot of oscillatory protein concentrations

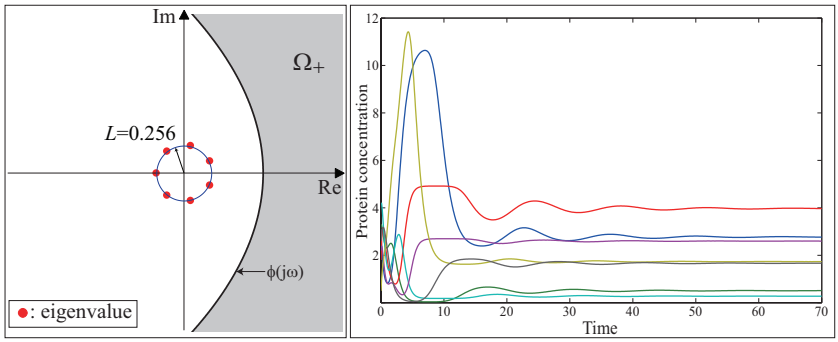


Figure 7: (Right) The domain Ω_+ and the eigenvalues of K: All eigenvalues are located outside Ω_+. (Left) Plot showing the convergence of protein concentrations to a unique equilibrium state

$-0.05, \xi_3 = -0.10, \xi_4 = -0.03, \xi_5 = -0.11, \xi_6 = -0.13$ and $\xi_7 = -0.05$. Then, the worst case parameters for the exsitence of periodic oscillations are obtained from Proposition 3 as $a = 1.5, b = 2.5, c = 8.0, \beta = 8.5$, which implies $R = 4.26$ and $Q = 0.968$.

Let the linearized homogeneous gene regulatory network system $\mathcal{H}(s)$ be defined by using the above (a, b, c, β) and ξ_i $(i = 1, 2, \cdots, 7)$. We first verify the effectiveness of the result in Theorem 1 (graphical criterion). The radius L of the eigenvalues of K is computed by (19) as $L = 1.32$. On the other hand, the domain Ω_+ is drawn based on (20) as illustrated in Fig. 6(Left). Since two eigenvalues belong to the domain Ω_+ in Fig. 6(Left), we can readily conclude that any of the heterogeneous gene regulatory network systems satisfying the above parameter bounds have periodic oscillations of protein concentrations. The same conclusion also immediately follows from Theorem 2, since $L = 1.32$ and $W(7, 0.968) = 1.06$.

To confirm the above result, let the actual parameters of the heterogeneous gene regulatory network be set as $\boldsymbol{a} = [1.2, 1.0, 1.2, 1.4, 1.0, 1.4, 1.2]^T$,

$\boldsymbol{b} = [1.4,\ 2.2,\ 1.5,\ 1.5,\ 1.4,\ 2.2,\ 2.2]^T$, $\boldsymbol{c} = [8.8,\ 8.1,\ 9.2,\ 9.2,\ 8.1,\ 8.2,\ 8.1]^T$, $\boldsymbol{\beta} = [8.9,\ 9.8,\ 8.7,\ 8.9,\ 9.8,\ 9.8,\ 8.7]^T$, where the i-th element of each vector denotes a_i, b_i, c_i and β_i, respectively. We remark that the actual values of ζ_i $(i = 1, 2, \cdots, 7)$ are obtained via numerical computation of the equilibrium as $\boldsymbol{\zeta} = [-0.102,\ -0.0706,\ -0.103,\ -0.0495,\ -0.121,\ -0.150,\ -0.0667]^T$. Then, the time course of simulated protein concentrations in the cyclic gene regulatory network is obtained as illustrated in Fig. 6(Right). The protein concentrations indeed exhibit periodic oscillations.

Example 2. In this example, we observe the contraposition of Theorem 1 and Theorem 2. The contraposition of the theorems states that if the protein concentrations converge to an equilibrium state for some heterogeneous gene regulatory network, the conditions of the theorems do not hold, *i.e,* all the eigenvalues of K lie outside of Ω_+, and $L < W(N, Q)$. Consider the cyclic gene regulatory network where there are $N = 7$ genes and all the interactions are repressive, *i.e.,* $f_i(\cdot) = F_R(\cdot)$ for $i = 1, 2, \cdots, 7$. Suppose the protein concentrations in the heterogeneous cyclic gene regulatory network converge to an equilibrium state as illustrated in Fig. 7(Right), and the parameters are identified as $\boldsymbol{a} = [1.0,\ 2.7,\ 2.7,\ 4.8,\ 4.8,\ 1.0,\ 2.7]^T$ $\boldsymbol{b} = [2.4,\ 2.0,\ 2.4,\ 2.0,\ 2.4,\ 2.4,\ 2.0]^T$ $\boldsymbol{c} = [5.0,\ 5.0,\ 5.8,\ 7.2,\ 5.8,\ 5.0,\ 7.2]^T$ $\boldsymbol{\beta} = [5.2,\ 5.2,\ 5.5,\ 7.0,\ 5.5,\ 7.0,\ 5.2]^T$. Let the linear system $\mathcal{H}(s)$ be defined with $a = 4.8(= \overline{a})$,$b = 2.4(\overline{b})$, $c = 5.0(= \underline{c})$, $\beta = 5.2(= \underline{\beta})$.

Let us verify the graphical criterion of Theorem 1. First, Q is calculated as $Q = 0.943$ and the region Ω_+ is drawn as Fig. 7(Left). On the other hand, R and ξ_i $(i = 1, 2, \cdots, 7)$ in L of (19) are computed as $R = 1.50$ and $\boldsymbol{\xi} = [-0.0713,\ -0.647,\ -0.0262,\ -0.446,\ -0.0847,\ -0.0221,\ -0.241]^T$, where ξ_i is exactly the same value as ζ_i of (6) since the values of ζ_i $(i = 1, 2, \cdots, 7)$ are exactly calculated from (a_i, b_i, c_i, β_i) $(i = 1, 2, \cdots, 7)$. Then, we observe that $L = 0.256$ and the eigenvalues of the matrix K are located outside of the region Ω_+. The above result is consistent with the Theorem 1. In addition, it also agrees with the analytic criterion of Theorem 2 since $L = 0.256$ and $W(7, 0.943) = 1.06$.

6 Analytic criterion and biological insights

6.1 Analaytic criterion involving equilibrium point analysis

In Theorem 1 and Theorem 2, we have assumed that the perturbed ranges of the linearized gains ξ_i of $f_i(\cdot)$ $(i = 1, 2, \cdots, N)$, are given in advance. However, it sometimes makes it difficult to unravel the relation between dynamical properties and the parameters of the gene regulatory network systems because ξ_i $(i = 1, 2, \cdots, N)$ are not the biological parameters. Thus, in this section, we derive the analytic criterion that does not depend on the linearized gains of $f_i(\cdot)$. In particular, it is indispensable to explicitly take the dependence of the equilibrium state on the parameters into account

because the linearized gain ζ_i depends on the equilibrium state. Thus, we first reveal the relation between the equilibrium point and the system's parameters in the following, though it is one of the challenging problems in nonlinear control systems analysis.

For the sake of analysis, we hereafter focus our attention to the class of homogeneous cyclic gene regulatory networks where all the interactions between genes are repressive, *i.e.,* $f_i(\cdot) = F_R(\cdot)$ for $i = 1, 2, \cdots, N$. Note that the result presented in this section can be directly applied to Repressilator (Elowitz and Leibler, 2000) since it was performed with three repressors interacting in a cyclic way.

Let $f(\cdot)$ be defined as $f(\cdot) := F_R(\cdot)(= f_1(\cdot) = f_2(\cdot) = \cdots = f_N(\cdot))$. It follows from (1) and the definition of equilibria that $p_i^* = R^2 f(p_{i-1}^*)$ for $i = 1, 2, \cdots, N$, where p_i^* is the value of an equilibrium state of p_i. Then, by repeatedly applying the above equation, we have (4). In particular, $p_1^* = p_2^* = \cdots = p_N^* (=: p^*)$ holds because of the symmetric property that replacing the index i and j $(i \neq j)$ in (1) does not affect the dynamics. Moreover, the symmetric property simplifies (4) as

$$p^* = R^2 f(p^*) = \frac{R^2}{1 + p^{*\nu}}. \tag{26}$$

Then, ζ_i in (6) can be expressed as follows.

Lemma 5. *Consider the cyclic gene regulatory networks modeled by (1). Suppose* $f_i(p_{i-1}) = 1/(1 + p_{i-1}^\nu)$ *for* $i = 1, 2, \cdots, N$. *Then,* ζ_i *in (6) is identical to each other, i.e.,* $\zeta_1 = \zeta_2 = \cdots = \zeta_N (=: \zeta)$. *Moreover, the following holds:*

$$\zeta = -|f'(p^*)| = -\frac{\nu}{R^4}(R^2 - p^*). \tag{27}$$

Proof is presented in Appendix C. In (27), ζ is written in linear form with respect to p^*, and this linearity plays a key role in the following analysis.

Consider the analytic criterion given in Theorem 2. First, L in the left-hand side of (22) can be simplified by Lemma 5 as $L = R^2|\zeta| = \nu(R^2 - p^*)/R^2$, and the condition in (22) can be rewritten as

$$\frac{p^*}{R^2} < 1 - \frac{W(N, Q)}{\nu} \tag{28}$$

Since the equilibrium protein concentration p^* satisfies $p^* > 0$, we have the following proposition.

Proposition 4. *Consider the homogeneous cyclic gene regulatory networks modeled by (1) where* $a_i = a, b_i = b, c_i = c, \beta_i = \beta$ $(i = 1, 2, \cdots, N)$. *Suppose* $f_i(p_{i-1}) = 1/(1 + p_{i-1}^\nu)$ *for* $i = 1, 2, \cdots, N$. *Then, the gene regulatory network has periodic oscillations of protein concentrations for some* R *if* $\nu > W(N, Q)$ *is satisfied.*

Proof. Suppose the right-hand side of (28) is positive, which is equivalent to the inequality in Proposition (4). First, it is important to note that the right-hand side of (28) depends only on (N, ν, a, b) because of the definition of Q in (21), and the left-hand side depends on (ν, a, b, c, β) because of the definition of $R(:= \sqrt{c\beta}/\sqrt{ab})$ and p^*. Consider some fixed values (N_0, ν_0, a_0, b_0). Then, the right-hand side of (28) is fixed, and only R in the left-hand side is a free parameter. We claim that p^*/R^2 monotonically decreases as R decreases, which follows from the argument below: If p^* does not decrease as R decreases, (26) does not hold because the right-hand side of (26) decreases while the left-hand side does not. In particular, $\lim_{R\downarrow 0} p^* \to 0$ follows from (26). Therefore, we can always find R which makes $\mathcal{H}(s)$ unstable by choosing sufficiently small c and β. □

Proposition 4 is the analytic condition for the existence of R which guarantees the existence of periodic oscillations. In particular, it is concluded from the statement that the existence of periodic oscillations is determined by using these four parameters (N, ν, R, Q), while the six parameters (N, ν, a, b, c, β) are given in advance. Hence, we hereafter concentrate on quantitative relation between these parameters and the existence of periodic oscillations.

Let ρ be defined by $\rho := 1 - W(N, Q)/\nu$. It follows from (26) that (28) can be equivalently written as $p^{*\nu+1} + p^* = R^2 < (R^2\rho)^{\nu+1} + R^2\rho$. with $p^* > 0$. Therefore, solving the above inequality leads the following analytic criterion for the existence of periodic oscillations which explicitly considers the relation between the equilibrium and the parameters.

Theorem 3. *Consider the homogeneous cyclic gene regulatory networks modeled by (1) where* $a_i = a, b_i = b, c_i = c, \beta_i = \beta$ $(i = 1, 2, \cdots, N)$. *Suppose* $f_i(p_{i-1}) = 1/(1 + p_{i-1}^{\nu})$ *for* $i = 1, \cdots, N$. *Then, there exist periodic oscillations of protein concentrations* $p_i (i = 1, 2, \cdots, N)$, *if both*

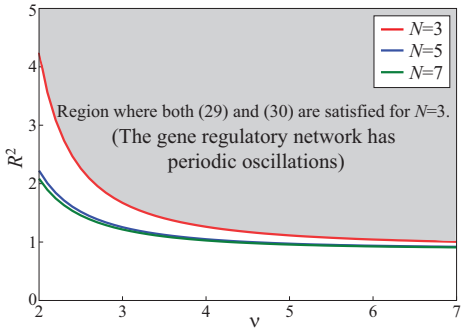
$$\nu > W(N, Q) \tag{29}$$

and

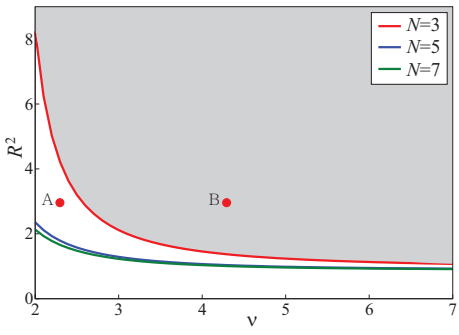
$$R^2 > \left(\frac{W(N, Q)}{\nu - W(N, Q)}\right)^{\frac{1}{\nu}} \left(\frac{\nu}{\nu - W(N, Q)}\right) \tag{30}$$

are satisfied, where $W(N, Q)$ *is defined by (23).*

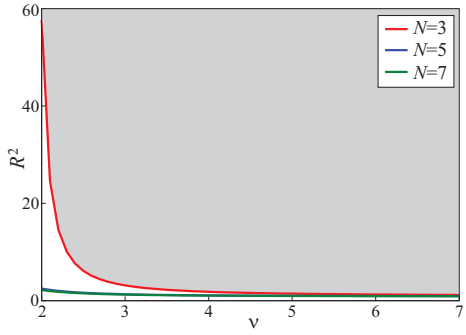
This analytic condition only depends on the given biochemical parameters (N, ν, a, b, c, β), and thus, we can easily discuss the relation between the parameters and the existence of periodic oscillations. In particular, it should be emphasized that (29) and (30) are written only in terms of the four quantities (N, ν, R, Q). This implies that these four parameters are essential for determining the existence of periodic oscillations..



(a) $(N, \nu, R^2, Q^2) = (*, *, *, 1.0)$



(b) $(N, \nu, R^2, Q^2) = (*, *, *, 0.5)$



(c) $(N, \nu, R^2, Q^2) = (*, *, *, 0.1)$

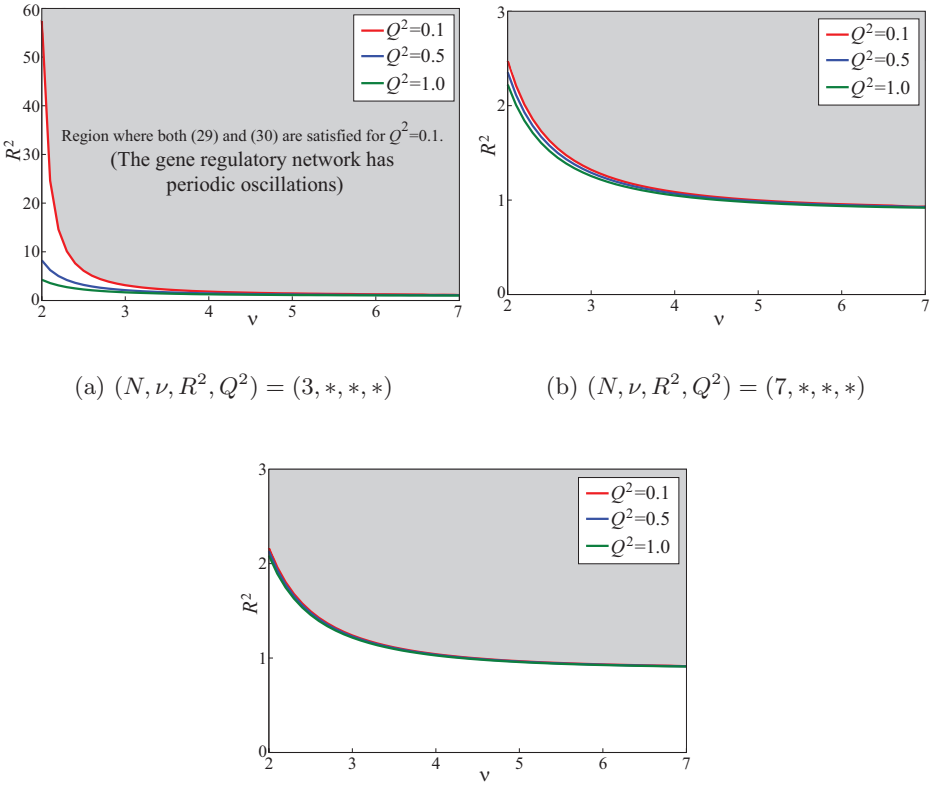
Figure 8: Parameter domain for the existence of periodic oscillations. For any Q, cyclic gene regulatory networks tend to have periodic oscillations as N and ν get larger.

We remark that Proposition 14 in Samad et al. (2005) gave the analytic criterion for the existence of oscillations for $N = 3$, which was developed based on direct computation of the eigenvalues of the Jacobian. It is obvious that Theorem 3 with $Q = 1$ (*i.e.*, $a = b$) and $N = 3$ corresponds to their result.

6.2 Biological insight

In this section, we give an interpretation of Theorem 2 from a biological viewpoint, and reveal the class of cyclic gene regulatory networks which tends to have periodic oscillations. Then, we perform illustrative numerical simulations to elucidate the insight.

First, we see from (29) and (30) that the four physical quantities (N, ν, R, Q) are essential to determine the existence of periodic oscillations. There-



(a) $(N, \nu, R^2, Q^2) = (3, *, *, *)$ (b) $(N, \nu, R^2, Q^2) = (7, *, *, *)$

(c) $(N, \nu, R^2, Q^2) = (11, *, *, *)$

Figure 9: Parameter domain for the existence of periodic oscillations. Cyclic gene regulatory networks tend to have periodic oscillations as Q gets close to 1.0 (*i.e.*, $T_r \simeq T_p$). Also, the effect of Q is ignorable when the number of genes, N, is sufficiently large.

fore, these four parameters may potentially become a clue to unravel the underlying principles of the dynamical properties in gene regulatory networks. In particular, the proposed parameters R and Q are interpreted as follows: R is the ratio between the geometric means of the degradation and production rates, and Q is the ratio between the arithmetic and geometric means of degradation rates (see (16) and (21)). In the sequel, we concentrate on how these four parameters affect the existence of periodic oscillations.

First, (29) should be naturally satisfied in practical gene regulatory networks, because it is equivalent to p^* satisfying (28) to be positive. Thus, it is inferred that the crucial condition is (30). In (30), the right-hand side monotonically decreases, as the number of genes, N gets larger. It means that the cyclic gene regulatory networks consisting of a large number of genes are more likely to have periodic oscillations. This fact is also confirmed from

Fig. 8 where the parameter region satisfying (30) for several values of Q is illustrated. Moreover, it may be concluded from Fig. 8 that the cyclic gene regulatory networks with a relatively large Hill coefficient, ν, tend to have periodic oscillations [2]. In addition, the right-hand side of (30) is a monotonically decreasing function with respect to Q, and thus, the existence of periodic oscillations is more likely to be expected as Q approaches to a unity, which means mRNA and protein lifetimes (*i.e.,* T_r and T_p) get closer. It can be confirmed from Fig. 9 as well, where each plot illustrates the parameter region satisfying (30) for various N. In particular, it is observed from Fig. 9 that the effect of Q can be disregarded when the number of genes, N, is large.

In summary, the cyclic gene regulatory network gets more likely to have periodic oscillations if some or all of the followings are satisfied:

- The number of genes, N, gets larger,
- The Hill coefficient, ν, gets higher,
- mRNA and protein lifetimes, T_r and T_p, get closer.

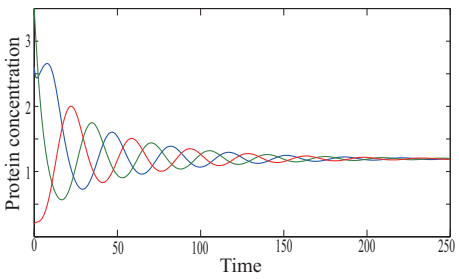
Example. To elucidate the biological insights obtained above, we will see some illustrative examples.

First, we consider the cyclic gene regulatory network composed of $N = 3$ genes where all interactions between genes are repressive. Suppose the parameters of the gene regulatory network become $a_1 = a_2 = a_3 = 1.00, b_1 = b_2 = b_3 = 0.172, c_1 = c_2 = c_3 = 0.360$ and $\beta_1 = \beta_2 = \beta_3 = 1.43$, and the Hill coefficient ν is $\nu = 2.3$. Then, it is easily verified that $R^2 = 3.0$ and $Q^2 = 0.50$. The point A in Fig. 8(b) corresponds to the above parameters, which does not guarantee the existence of periodic oscillations. Indeed, the numerical simulation result in Fig. 10(a) shows convergence to an equilibrium state.

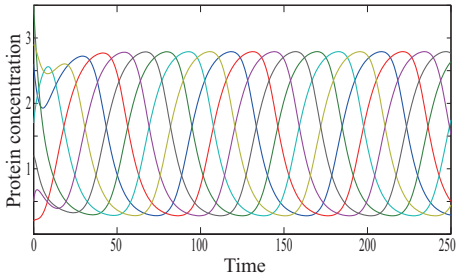
According to the above insight, the cyclic gene regulatory networks are more likely to have periodic oscillations as the number of genes gets larger. Thus, in the next example, we set the parameters a_i, b_i, c_i, β_i and ν to the same ones in the above example, and just change the number of genes to $N = 7$. The time course of simulated protein concentrations is illustrated in Fig. 10(b), which shows periodic oscillations. In fact, this example corresponds to the point A with $N = 7$ in Fig. 8(b), which implies the existence of periodic oscillations.

Finally, we see another example to confirm the insight that the Hill coefficient ν should be large for the existence of periodic oscillations. Let the number of genes be $N = 3$, and a_i, b_i, c_i and β_i are set to the same values as in the first example. Since the time plot of the protein concentration shows

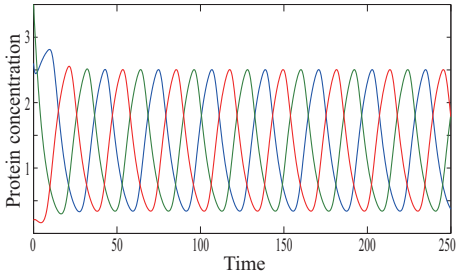
[2]Note that this is not necessarily the case for sufficiently large ν which is outside of the range of our interest.



(a) Case A with $N = 3$



(b) Case A with $N = 7$



(c) Case B with $N = 3$

Figure 10: Time plot of protein concentrations: the gene regulatory network tends to have periodic oscillations as the number of genes and the Hill coefficient get large.

the convergence in the first example with $\nu = 2.3$, we here consider the case of $\nu = 4.3$. In this case, the time plot of protein concentrations exhibits periodic oscillations as shown in Fig. 10(c). This example corresponds to the point B in Fig. 8(b), and the existence of periodic oscillations is indeed predicted.

Note that we do not rely on numerical computation to obtain the parameter region such as Fig. 8 and Fig. 9, because the criterion is obtained in analytic form as shown in (30). Though there are many previous works that rely on numerical computations, such approaches may suffer from the potential drawback that the essential physical quantities such as R and Q are difficult to obtain. On the other hand, analytic criteria obtained in this paper can give biological insights, and are helpful for understanding the underlying essence of biochemical networks.

7 Discussion on effects of time delay

Though the model in (1) is broadly accepted, it is said that sizable time delays in the transportation of chemical substances in a cell play a significant

role to determine dynamical behavior of proteins. In particular, such effect is unignorable for eukaryotic cells, because the volume of the cell is relatively large, and mRNA and protein productions occur at different locations (Chen and Aihara, 2002). Here, we briefly discuss the effect of such time delay.

Cyclic gene regulatory networks with time delay are modeled as, for $i = 1, 2, \cdots, N$,

$$\begin{aligned} \dot{r}_i(t) &= -a_i r_i(t) + \beta_i f_i(p_{i-1}(t - \tau_{p_{i-1}})), \\ \dot{p}_i(t) &= c_i r_i(t - \tau_{r_i}) - b_i p_i(t), \end{aligned} \tag{31}$$

where τ_{p_i} and τ_{r_i} are the time delays of transcription and translation of the i-th gene, respectively (Chen and Aihara, 2002). In this case, Poincaré-Bendixson type theorem for cyclic feedback systems with delay (Mallet-Paret and Sell, 1996), which is similar to Proposition 1, is available [3]. Then, it is concluded that the cyclic gene regulatory network system with delay in (31) has oscillations if the equilibrium point is locally unstable.

The overall dynamics of the linearized model of homogeneous gene regulatory network is obtained in a similar way to (17) as

$$\mathcal{H}_{\mathcal{T}}(s) = \left(\phi(s) e^{s\mathcal{T}} I - K\right)^{-1}, \tag{32}$$

where $\mathcal{T}$ is the average time delay, $\mathcal{T} := \sum_{i=1}^{N} (\tau_{r_i} + \tau_{p_i})/N$, and K and $\phi(s)$ are as defined in (15) and (17), respectively. Note that during the derivation, we employ the distributive property, and equally distribute the time delays among $h(s)$. The stability of $\mathcal{H}_{\mathcal{T}}(s)$ can be analyzed in a similar fashion to $\mathcal{H}(s)$, and the following graphical criterion for the existence of oscillations is obtained.

Theorem 4. *Consider the heterogeneous cyclic gene regulatory networks modeled by (31). Then, if at least one of the eigenvalues of K lies inside the domain Ω_+ defined by*

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

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

An example of the region Ω_+ is illustrated in Fig. 11(right). It is observed that the boundary of Ω_+, *i.e.,* $\phi(j\omega) e^{j\omega\mathcal{T}}$, is obtained by rotating each point of $\phi(j\omega)$ by $\omega\mathcal{T}$ (see Fig 11(right)). Then, it can be proven that the eigenvalues of K at $\theta = \pm\pi/N$ always cross the boundary first. Furthermore, the equilibrium point and its properties shown in Section 6 do not change by the time delays. Hence, the following analytic criterion for the existence of oscillations is obtained in a similar fashion to Theorem 3 (see Appendix D for proof).

[3] Unlike Proposition 1, homoclinic oscillations are not ruled out in the discussion of this section.

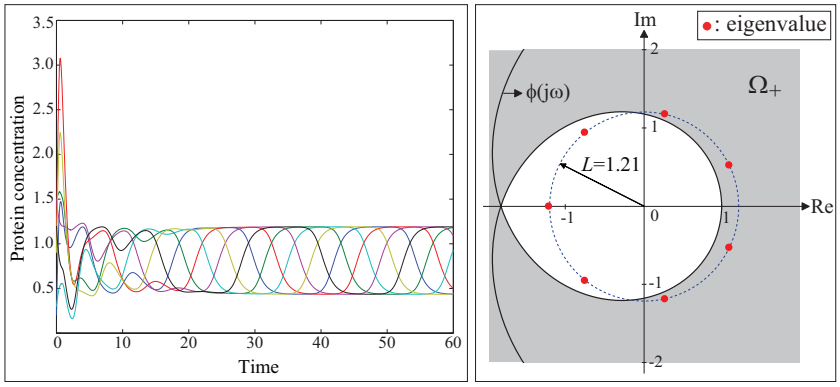


Figure 11: (Left)Time plot of oscillatory protein concentrations in a cyclic gene regulatory network with time delay. (Right) The domain Ω_+ and the eigenvalues of K: Two eigenvalues belong to Ω_+.

Theorem 5. *Consider the homogeneous cyclic gene regulatory networks modeled by (31) where* $a_i = a, b_i = b, c_i = c, \beta_i = \beta$ $(i = 1, 2, \cdots, N)$. *Suppose* $f_i(p_{i-1}) = 1/(1 + p_{i-1}^{\nu})$ *for* $i = 1, \cdots, N$.

(i) If both (29) and (30) hold, there exist oscillations of protein concentrations p_i, *for any* $\tau_{r_i} \geq 0$ *and* $\tau_{p_i} \geq 0$ $(i = 1, \cdots, N)$,

(ii) else if both $\nu > 1$ *and*

$$R^2 > \left(\frac{1}{\nu - 1}\right)^{\frac{1}{\nu}} \left(\frac{\nu}{\nu - 1}\right) \tag{33}$$

hold, then there exist critical time delays $\tau_{r_i}^* > 0$ *and* $\tau_{p_i}^* > 0$ $(i = 1, \cdots, N)$ *such that the cyclic gene regulatory network has oscillations when* $\tau_{r_i} > \tau_{r_i}^*$ *and* $\tau_{p_i} > \tau_{p_i}^*$.

The case (i) in the above theorem means that if there exist periodic oscillations in a cyclic gene regulatory network without delay, the existence of oscillations is also guaranteed in this network system with any time delays. On the other hand, (ii) means that even if the existence of oscillations is not guaranteed in a cyclic gene regulatory network without delay, it can be guaranteed when the time delays are sufficiently large. Otherwise, it can be stated that the equilibrium point is locally stable, though it does not necessarily imply nonexistence of oscillations.

It should be noted that Theorem 5 encompasses the discussion presented in Section IV in Chen and Aihara (2002), because our criterion explicitly considers the change of the equilibrium point with respect to parameters, and does not assume that the equilibrium point is given in advance.

8 Conclusion

In this paper, we have studied criteria for the existence of periodic oscillations in cyclic gene regulatory networks. First, we have shown that the existence of periodic oscillations in heterogeneous gene regulatory networks can be checked by local instability analysis of homogeneous gene regulatory networks. Then, the graphical criterion was presented based on the Poincaré-Bendixson type theorem (Mallet-Paret and Smith, 1990) and the local instability analysis of the linearized gene regulatory network system. Based on this criterion, the analytic criterion was derived, and the relation between biochemical parameters and the equilibrium point was clarified. Although the equilibrium point of the cyclic gene regulatory networks depends on the system's parameters, many existing works do not take such effects into account. In contrast, the presented analytic criterion has the distinctive features that (i) it explicitly considers the relation between the equilibrium point and the parameters, (ii) it is written only in terms of given biochemical parameters, and (iii) it is applicable to cyclic gene regulatory networks composed of any number of genes. Thus, it is easy to interpret the result from a biological viewpoint. In fact, we have provided novel biological insights on the class of cyclic gene regulatory networks that is more likely to have periodic oscillations based on the analytic result. Finally, we have discussed the case where unignorable time delay exists in the dynamics of gene expression. In short, the most fundamental yet essential gene regulatory networks have been comprehensively analyzed in this paper. Thus, our next step will be to introduce additional interactions between genes, and develop a more versatile analysis method for such networks.

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A Proof of Lemma 2

First, we see that $(R_i^2 f_i) \circ (R_{i-1}^2 f_{i-1})(p_{i-2})$ in (4) becomes a monotonic function, *i.e.,* it is a monotonically increasing function when $f_i'(\cdot) \cdot f_{i-1}'(\cdot) > 0$, and a decreasing function when $f_i'(\cdot) \cdot f_{i-1}'(\cdot) < 0$. Then, by applying the above argument recursively, we see from Assumption 1 that the right-hand side of (4) decreases monotonically, Therefore, the existence of a unique solution p_i^* $(i = 1, 2, \cdots, N)$ follows from the monotonicity of both sides of (4). Uniqueness of r_i^* $(i = 1, 2, \cdots, N)$ is clear because $r_i^* = b_i p_i^* / c_i$ holds from the definition.

B Proof of Lemma 3

First, Jacobian can be written as

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

Then, it is easily verified that $\det(-J) = \prod_{i=1}^{N} a_i b_i - \prod_{i=1}^{N} c_i \beta_i f_i'(p_{i-1}^*)$. Assumption 1 implies that the second term of the right-hand side of $\det(-J)$ becomes negative, which implies $\det(-J) > 0$

C Proof of Lemma 5

The former part of the claim is clear because $p_1^* = p_2^* = \cdots = p_N^*$ follows from the symmetric property, In the following, we prove the latter part. It follows from the definition of $f(\cdot)$ that $\zeta = -|f'(p^*)| = -\nu p^{*\nu-1}/(1+p^{*\nu})^2$. Then, by repeatedly applying (26), we have

$$-\frac{\nu p^{*\nu-1}}{(1+p^{*\nu})^2} = -\frac{\nu p^{*\nu+1}}{R^4} = -\frac{\nu}{R^4}(R^2 - p^*). \tag{34}$$

D Proof of Theorem 5

The eigenvalues of K at $\theta = \pm\pi/N$ always pass through the boundary of Ω_+ in Fig. 11(Right) first, because of the monotonicity of $|\phi(j\omega)|$ and $\arg(\phi(j\omega))$ mentioned in the proof of Theorem 2. Then, the statement (i) in Theorem 5 immediately follows from Theorem 4, because the conditions (29) and (30) in Theorem 4 are equivalent to that the eigenvalues of K at $\theta = \pm\pi/N$ lie inside Ω_+ with $\mathcal{T} = 0$.

The statement (ii) in Theorem 5 is equivalent to that the radius of the circle where all the eigenvalues of K are located is greater than the minimum distance between the origin and the boundary of Ω_+. Thus, the statement (ii) follows from the fact that $|\phi(j\omega)e^{j\omega\tau}| = |\phi(j\omega)|$ holds, and the minimum distance is given as $\phi(0) = 1$.