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Time Delay Effects on Oscillation Profiles in Cyclic Gene Regulatory Networks: Harmonic Balance Approach

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Abstract— **Oscillatory gene expression is closely connected to periodic physiological functions. In this paper, we provide a systematic method to study the oscillation profiles of gene expression in large-scale cyclic genetic regulatory networks based on multivariable harmonic balance. In particular, we here turn our attention to time delay in transcription and translation process, and analytically derive the relation between biological parameters and the frequency, phase and amplitude of the oscillations. Then, the roles of the time delay are revealed.**

I. INTRODUCTION

Oscillatory chemical reactions in genetic regulatory networks are closely related to rhythmic bodily functions such as circadian rhythms [1]. In particular, quantitative properties of the rhythms are mostly connected to the period and the phase of the oscillations of protein levels [1], which are robustly regulated by feedback mechanisms in living cells.

Recent studies showed that the period of oscillatory gene expression ranges widely from minutes to hours depending on the reactions, and the phase difference between protein species also plays an important role in producing circadian clocks (see [1] and references therein). However, the precise mechanisms regulating such oscillation patterns are still to be resolved. Hence, this paper aims to develop a theoretic approach for quantifying the oscillation profiles of gene expression.

The cyclic network motif, where each gene activates or represses another gene expression in a cyclic way as shown in Fig. 1, is one of the fundamental structures that can exhibit periodic oscillations, and it is embedded in large-scale gene regulatory networks. During the last few decades, many works have been devoted to study the dynamical properties of the cyclic gene regulatory network [2]–[5].

In particular, Repressilator [4], the engineered genetic oscillator, triggered the several works [5]–[7], which were concerned with oscillation profile analysis with engineering tools. In [5], frequency of oscillatory gene expression was numerically examined. The result was consistent with the qualitative insight obtained from [8], where a harmonic balance technique was applied to the Goodwin oscillator [2]. On the other hand, the phase difference between protein species was studied in [6] by phase space analysis. Recently, the authors [9] presented a systematic approach for studying frequency, phase and amplitude of the oscillations using the idea of the multivariable harmonic balance [10]. Then, relations of biological parameters and the oscillation profiles were analytically obtained.

In these previous works, however, the inherent time delays in transcription, translation and translocation process in gene regulatory networks have been neglected. 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 [11]. Thus, the time delay has to be explicitly considered to gain more general and reliable biological insight into the regulation mechanisms of the oscillations.

In this paper, we consider the cyclic gene regulatory networks with time delay. Our goal is to reveal relations of biochemical parameters and the profiles of oscillations in an analytic way. Specifically, frequency, phase and amplitude of oscillations are studied with the harmonic balance method [12], which is one of classical frequency domain techniques to examine nonlinear oscillatory behaviors with a certain approximation of the waveform of the system's output. Since the non-delay case was extensively studied in the authors' previous work [9], we here turn our attention to time delay, and extend the previous analysis scheme. Then, the potential roles of the time delay are discussed based on our analytic results.

This paper is organized as follows. Section II provides the dynamical model of the cyclic gene regulatory networks. In Section III, we introduce theoretical framework of the multivariable harmonic balance. Then, the main results, *i.e.,* analytic estimates of oscillation profiles of protein levels, are presented in Section IV. Section V argues effects of time delay, and it is confirmed by illustrative numerical simulations. Finally, Section VI concludes this paper.

II. MODEL AND EXISTENCE OF OSCILLATIONS

A. Dynamical Model of cyclic gene regulatory networks with time delay

The gene regulatory networks, where each protein activates or represses another transcription in a cyclic way as illustrated in Fig. 1, are called cyclic gene regulatory networks. The dynamics of mRNA and protein concentrations in the cyclic gene regulatory networks consisting of N genes is modeled by the following delay differential equations [11]:

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

for $i = 1, 2, \cdots, N$, where $r_i \in \mathbb{R}_+ (:= \{x \in \mathbb{R} \mid x \geq 0\})$ and $p_i \in \mathbb{R}_+$ denote the concentrations of the i-th mRNA

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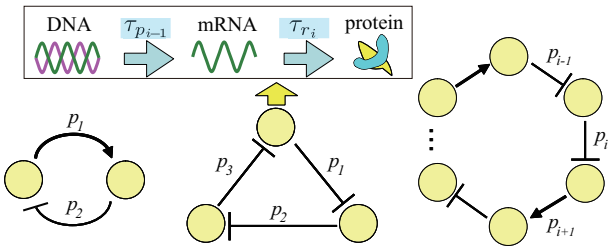

Fig. 1. Gene regulatory networks with negative cyclic feedback. The symbols $\rightarrow$ and $\dashv$ represent activation and repression of transcription, respectively. (Left) activator-repressor motif, (Center) successive repressor motif, or Repressilator motif [4], (Right) generic negative cyclic motif considered in this paper.

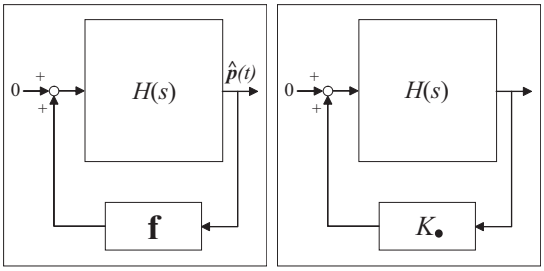

Fig. 2. (Left) block diagram of negative cyclic gene regulatory networks, (Right) linear system $\mathcal{H}_\bullet(s)$ in (14). The static nonlinearity $\mathbf{f}$ is replaced with the corresponding describing function.

and its corresponding protein synthesized by the i-th gene, respectively. Let the subscript 0 be replaced by N throughout this paper for the sake of notational simplification. Positive constants τ_{r_i} and τ_{p_i} denote time delay in transcription and translation process, respectively, and the kinetic 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. The nonlinear function $f_i(\cdot) : \mathbb{R}_+ \rightarrow \mathbb{R}_+$ stands for the effect of either activation or repression of the transcription, and it is a single-valued monotone function satisfying $f_i(0) = 1$ and $f_i(\infty) = 0$ for repression and $f_i(0) = 0$ and $f_i(\infty) = 1$ for activation.

Suppose $a_1 = a_2 = \cdots = a_N (=: a)$ and $b_1 = b_2 = \cdots = b_N (=: b)$ in (1). Then, the overall dynamics of gene regulatory network systems defined by (1) can be formulated by a transfer matrix $H(s)$ and a static vector nonlinearity function $\mathbf{f}$ as shown in Fig. 2 (Left), where

$$H(s) := \mathrm{diag}(h_1(s), h_2(s), \cdots, h_N(s)), \tag{2}$$

$$\mathbf{f} := [R_1^2 f_1(\cdot), R_2^2 f_2(\cdot), \cdots, R_N^2 f_N(\cdot)]^T \tag{3}$$

with

$$h_i(s) := \frac{e^{-s(\tau_{r_i}+\tau_{p_i})}}{(T_a s+1)(T_b s+1)},\ T_a := \frac{1}{a},\ T_b := \frac{1}{b}, \tag{4}$$

$$R_i := \frac{\sqrt{c_i \beta_i}}{\sqrt{ab}}.\quad (i = 1, 2, \cdots, N). \tag{5}$$

B. Existence of oscillations

It is known that dynamical behavior of the system (1) is characterized by

$$\delta := \left(\frac{df_1}{dp}\right) \cdot \left(\frac{df_2}{dp}\right) \cdots \left(\frac{df_N}{dp}\right). \tag{6}$$

Specifically, the protein concentrations asymptotically converge to one of equilibria when $\delta > 0$, while they exhibit oscillatory behaviors as well as convergence when $\delta < 0$ [13]. Therefore, the following assumption is imposed to study the oscillation profiles in this paper.

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

This assumption implies that a given cyclic gene regulatory network has an odd number of repressive interactions ($df_i/dp < 0$) between genes.

TABLE I

PHYSICAL MEANINGS OF THE CONSTANTS

N	The number of genes in gene regulatory network
Q	Discrepancy of mRNA and protein degradation time
R_ℓ	Ratio of degradation and production rates, which accounts for equilibrium concentrations
$\hat{\tau}$	Normalized average time delay
ν	Hill coefficient, which quantifies the degree of cooperative binding

Existence conditions of oscillations were analytically obtained in Takada *et al.* [14] based on the analysis scheme shown in [15]. In particular, it was shown that five dimensionless quantities are essentially contributed to determine the existence of oscillations, namely $(N, Q, R_\ell, \hat{\tau}, \nu)$ $(\ell = 1, 2, \cdots, N)$, where

$$Q := \frac{\sqrt{T_a T_b}}{(T_a + T_b)/2}, \hat{\tau} := \frac{\tau}{\sqrt{T_a T_b}}, \tau := \frac{\sum_{i=1}^N (\tau_{r_i} + \tau_{p_i})}{N}, \tag{7}$$

and ν is the Hill coefficient (see [14] for details), which determines the degree of the nonlinearity $f_i(\cdot)$. Physical meanings of these constants are summarized in Table I. Note that $0 < Q \leq 1$ holds from the definition, and $Q \rightarrow 1$ as T_a and T_b tends to a same value.

Remark 1. In [14], $\tilde{\tau} := \frac{\tau}{(T_a+T_b)/2}$, instead of $\hat{\tau}$, was mainly used to interpret their result. However, we hereafter use $\hat{\tau}$, which can be easily obtained by Q and $\tilde{\tau}$ as $\hat{\tau} = \tilde{\tau}/Q$.

In the following, we assume the existence of oscillations, and consider the relation of the above constants and the oscillation profiles of protein levels in large-scale cyclic gene regulatory networks. In particular, we mainly turn our attention to the effect of the time delay, since the non-delay case was already studied in the authors' previous work [9]. The problem can be summarized as follows.

Problem. *Consider the cyclic gene regulatory networks with time delay modeled by (1). Predict the oscillation profiles, i.e., the frequency, phase and amplitude, of the oscillatory protein concentrations $p_i(t)$ $(i = 1, 2, \cdots, N)$ in an analytic way. Then, find biological insight into the relation between the biochemical parameters and the oscillation profiles.*

III. Framework of Oscillation Profile Analysis

In this section, we provide a framework of oscillation profile analysis. We first derive quasi-linear systems associated with (1) by approximating the oscillatory waveform of protein levels $p_i(t)$ and the nonlinearity $f_i(\cdot)$ $(i = 1, 2, \cdots, N)$ of the system. Then, we show the theoretical foundation of the oscillation profile analysis based on the approximation.

Let the waveform of $p_i(t)$ be approximated by

$$p_i(t) \simeq x_i + y_i \sin(\varpi t + \varphi_i) \quad (i = 1, 2, \cdots, N), \tag{8}$$

where $x_i > 0$ and $y_i > 0$ denote the bias and the amplitude of the first order harmonic components of the i-th protein $p_i(t)$, respectively, and ϖ and φ_i are the frequency and the relative phase between proteins, respectively. Then, the nonlinear function $f_i(p_{i-1}(t))$ can be approximated by its describing functions [12]:

$$\eta_i(x_{i-1}, y_{i-1}) := \frac{R_i^2}{2\pi x_{i-1}} \int_{-\pi}^{\pi} f_i\left(x_{i-1} + y_{i-1}\sin(t)\right) dt. \tag{9}$$

$$\xi_i(x_{i-1}, y_{i-1}) := \frac{R_i^2}{\pi y_{i-1}} \int_{-\pi}^{\pi} f_i\left(x_{i-1} + y_{i-1}\sin(t)\right) \sin(t) dt \tag{10}$$

The describing functions $\eta_i(x_{i-1}, y_{i-1})$ and $\xi_i(x_{i-1}, y_{i-1})$ represent the gains of $R_i^2 f_i(\cdot)$ for the bias and harmonic components, respectively, when the input is the biased sinusoidal $x_{i-1} + y_{i-1}\sin(\varpi t)$. Note that the describing functions are independent of ϖ when $f_i(\cdot)$ is a single-valued function.

Consequently, the closed loop equations that $\boldsymbol{x}$ and $\boldsymbol{y}$ are expected to satisfy for the quasi-linear system are obtained as

$$\begin{aligned} \left(I - H(0)\mathcal{K}_0(\boldsymbol{x}, |\boldsymbol{y}|)\right)\boldsymbol{x} &= 0, \\ \left(I - H(j\varpi)\mathcal{K}_1(\boldsymbol{x}, |\boldsymbol{y}|)\right)\boldsymbol{y} &= 0, \end{aligned} \tag{11}$$

where $\mathcal{K}_0(\boldsymbol{x}, |\boldsymbol{y}|) := \mathrm{cyc}(\eta_1, \eta_2, \cdots, \eta_N)$ and $\mathcal{K}_1(\boldsymbol{x}, |\boldsymbol{y}|) := \mathrm{cyc}(\xi_1, \xi_2, \cdots, \xi_N)$ with

$$\mathrm{cyc}(z_1, z_2, z_3, \cdots, z_N) := \begin{bmatrix} 0 & 0 & 0 & \cdots & z_1 \\ z_2 & 0 & 0 & \ddots & 0 \\ 0 & z_3 & 0 & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 0 & z_N & 0 \end{bmatrix}.$$

The symbols $\boldsymbol{x}$ and $\boldsymbol{y}$ are defined as $\boldsymbol{x} := [x_1, x_2, \cdots, x_N]^T \in \mathbb{R}_+^N$ and $\boldsymbol{y} := [y_1 e^{j\hat{\varphi}_1}, y_2 e^{j\hat{\varphi}_2}, \cdots, y_N e^{j\hat{\varphi}_N}]^T \in \mathbb{C}^N$ with

$$\hat{\varphi}_i := \varphi_i - \varpi\tau_{p_i}, \tag{12}$$

and $|\boldsymbol{y}|$ stands for elementwise absolute values, *i.e.,* $|\boldsymbol{y}| = [y_1, y_2, \cdots, y_N]^T \in \mathbb{R}_+^N$. It should be noted that $\hat{\boldsymbol{p}} := [\hat{p}_1(t), \hat{p}_2(t), \cdots \hat{p}_N(t)]^T$ in Fig. 2 (Left) can be written as

$$\hat{p}_i(t) = p_i(t - \tau_{p_i}) \simeq x_i + y_i \sin(\varpi t + \hat{\varphi}_i) \tag{13}$$

for $i = 1, 2, \cdots, N$. Therefore, the oscillation profile analysis reduces to the problem of finding $3N$ variables $(\varpi, x_1, x_2, \cdots, x_N,\ y_1, y_2, \cdots, y_N,\ \varphi_2, \varphi_3, \cdots, \varphi_N)$ satisfying (11), which is equivalent to finding a solution $(\varpi, \boldsymbol{x}, \boldsymbol{y})$ of (11). Note that φ_1 can be taken arbitrarily without loss of generality. We designate the first and the second equations in (11) as *bias* and *harmonic balance equations*, respectively.

Let $\boldsymbol{x}^*$ and $\boldsymbol{y}^*$ denote the constant vectors that satisfy the bias and the harmonic balance equations simultaneously. Define the linear systems $\mathcal{H}_0(s)$ and $\mathcal{H}_1(s)$ as

$$\mathcal{H}_\bullet(s) := (I - H(s)K_\bullet)^{-1} \quad (\bullet = 0, 1), \tag{14}$$

where $K_\bullet$ is the constant matrices defined by $K_\bullet := \mathcal{K}_\bullet(\boldsymbol{x}^*, |\boldsymbol{y}^*|)$ $(\bullet = 0, 1)$. The systems $\mathcal{H}_\bullet(s)$ $(\bullet = 0, 1)$ are obtained by replacing the nonlinearity $f_i(\cdot)$ with the constant gain computed from the describing functions. (see Fig. 2 (Right)) Thus, the associated linear system $\mathcal{H}_\bullet(s)$ contains some information on the oscillations of the original nonlinear system. Following the idea in Iwasaki [10], we suppose the predicted oscillation $(\varpi, \boldsymbol{x}^*, \boldsymbol{y}^*)$ is orbitally stable if both $\mathcal{H}_0(s)$ and $\mathcal{H}_1(s)$ are marginally stable with the poles of $s = 0$ and $s = \pm j\varpi$ on the imaginary axis, and the rest in the open left half plane, respectively. Consequently, the problem of oscillation profile analysis addressed in Section II-B can be summed up in the following proposition.

Proposition 1. *Consider the gene regulatory networks with time delay modeled by (1). Suppose there exist $(\varpi, \boldsymbol{x}, \boldsymbol{y})$ satisfying (11). Then, the oscillatory protein concentrations $p_i(t)$ are expected at frequency ϖ, with phase φ_i, bias x_i and amplitude y_i, i.e.*

$$p_i(t) \simeq x_i + y_i \sin(\varpi t + \varphi_i) \tag{15}$$

for $i = 1, 2, \cdots, N$, where ϖ, φ_i, x_i and y_i satisfy both of the following conditions: (i) The equation (11) holds, and (ii) $\mathcal{H}_\bullet(s)$ $(\bullet = 0, 1)$ are marginally stable.

Major differences from the non-delay case [9] are that the diagonal entries of $H(s)$ is no longer homogeneous due to the heterogeneous time delays, and $\boldsymbol{y}$ depends not only on φ_i but also on ϖ. Thus, the analysis is not as straightforward as the non-delay case [9].

The existence of the solution $(\varpi, \boldsymbol{x}, \boldsymbol{y})$ of (11) is probable when the waveform of the oscillations is sufficiently similar to the biased sinusoidal of (15). Hence, we hereafter assume the existence of a solution in the bias and the harmonic balance equations, and concentrate on the oscillation profile analysis.

IV. Main result

In this section, we analytically derive profiles of the oscillatory protein concentrations in terms of the biological parameters by solving the bias and the harmonic balance equations. It is assumed in this section that the system (1) has an oscillatory solution, and the bias and the harmonic balance equations have the solution $(\varpi, \boldsymbol{x}, \boldsymbol{y})$ that satisfies both (i) and (ii) in Proposition 1.

A. Preliminaries

We here show that the solution of the bias and harmonic balance equations is associated with eigenvalues and eigenvectors of certain matrices, which becomes the foundation of our analysis.

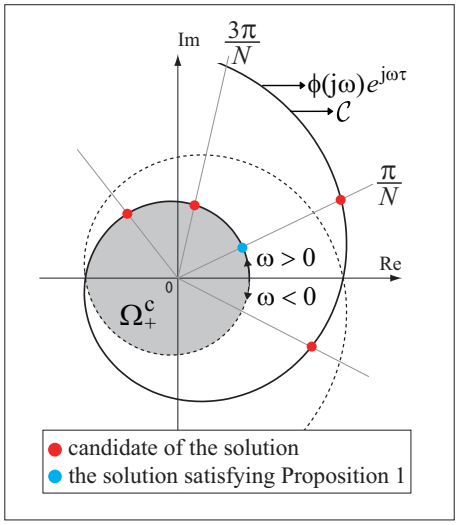

Fig. 3. Graphical interpretation of the harmonic balance equation. The red points satisfy the bias and the harmonic balance equations, but does not satisfy the marginal stability condition.

Let $N \times N$ transfer matrix $U(s)$ be defined by

$$U(s) := \mathrm{diag}(e^{-s(\tau-\tau_1)}, e^{-s(\tau-\tau_2)}, \cdots, e^{-s(\tau-\tau_N)}), \quad (16)$$

where $\tau_i := \tau_{r_i} + \tau_{p_i}$ $(i = 1, 2, \cdots, N)$. Then, $H(s)U(s) = h(s)e^{-s\tau}I$ holds, where

$$h(s) := \frac{1}{(T_a s + 1)(T_b s + 1)}, \ \phi(s) := \frac{1}{h(s)}. \quad (17)$$

Dividing (11) by $h(s)e^{-s\tau}$ yields another expression of the bias and the harmonic balance equations:

$$\begin{aligned} &(\phi(0)I - \mathcal{K}_0(\boldsymbol{x}, |\boldsymbol{y}|))\boldsymbol{x} = 0, \\ &(\phi(j\varpi)e^{j\varpi\tau}I - U^{-1}(j\varpi)\mathcal{K}_1(\boldsymbol{x}, |\boldsymbol{y}|))\boldsymbol{y} = 0. \end{aligned} \quad (18)$$

In (18), $\phi(0)$ and $\phi(j\varpi)e^{j\varpi\tau}$ correspond to eigenvalues of the matrix $K_0 (= \mathcal{K}_0(\boldsymbol{x}^*, |\boldsymbol{y}^*|))$ and $U^{-1}(j\varpi)K_1 (= U^{-1}(j\varpi)\mathcal{K}_1(\boldsymbol{x}^*, |\boldsymbol{y}^*|))$, respectively, while $\boldsymbol{x}^*$ and $\boldsymbol{y}^*$ become the corresponding eigenvectors. Hence, the solution of the bias and the harmonic balance equations is associated with the eigenvalues and eigenvectors of the matrices K_0 and $U^{-1}(j\varpi)K_1$.

In what follows, we predict the oscillation profiles using the property of the equations shown above.

B. Estimation of frequency

It follows from (18) that the expected frequency ϖ satisfies the eigenvalue equation $U^{-1}(j\varpi)K_1\boldsymbol{y}^* = \phi(j\varpi)e^{j\varpi\tau}\boldsymbol{y}^*$. Our goal in this section is to obtain ϖ that satisfies the equation.

Although ϖ appears in both sides of the eigenvalue equation, the following lemma shows that the eigenvalues of $U^{-1}(j\varpi)K_1$ do not depend on ϖ (see [16] for the proof).

Lemma 1. For any given $(\varpi, \boldsymbol{x}, \boldsymbol{y})$, the eigenvalues λ_i $(i = 1, 2, \cdots, N)$ of the matrix $U^{-1}(j\varpi)\mathcal{K}_1(\boldsymbol{x}, |\boldsymbol{y}|)$ are given by

$$\lambda_i := \left| \prod_{k=1}^{N} \xi_k(x_{k-1}, y_{k-1}) \right|^{\frac{1}{N}} e^{j\frac{2i-1}{N}\pi}. \quad (19)$$

We see that the eigenvalues of $U^{-1}(j\varpi)K_1$ coincide with the eigenvalues of K_1. Therefore, the solution of (18) can be obtained by solving $\phi(j\varpi)e^{j\varpi\tau} = \lambda_i$ $(i = 1, 2, \cdots, N)$.

This observation leads to the following graphical interpretation. The solution of $(\varpi, \boldsymbol{x}^*, \boldsymbol{y}^*)$ of (18) is given by the intersection of the vector locus $\mathcal{C} := \{\phi(j\omega)e^{j\omega\tau} \mid \omega \in \mathbb{R}\}$ and λ_i. In particular, Lemma 1 implies that the eigenvalues λ_i $(i = 1, 2, \cdots, N)$ are located on a circle, and the angular position of λ_i depends on neither $\boldsymbol{x}$ nor $\boldsymbol{y}$. Thus, all possible solutions of (18) are given by the intersections of the N straight lines $\{re^{j\frac{(2k-1)\pi}{N}} \mid r \geq 0, \ k = 1, 2, \cdots, N\}$ and the vector locus $\mathcal{C}$ (see Fig. 3).

Note that orbitally unstable solutions are ruled out by the marginal stability condition in Proposition 1 among the intersections. The following lemma relates the marginal stability of $\mathcal{H}_\bullet(s)$ and the above graphical interpretation (see [16] for the proof).

Lemma 2. The system $\mathcal{H}_\bullet(s)$ $(\bullet = 0, 1)$ defined by (14) has at least one pole on the imaginary axis and the rest in the open left half plane, if and only if at least one eigenvalue of $K_\bullet$ lies on the curve $\mathcal{C} = \{\phi(j\omega)e^{j\omega\tau} \mid \omega \in \mathbb{R}\}$ and the rest lies inside the open set Ω_+^c, where $\Omega_+^c := \{\gamma \in \mathbb{C} \mid \phi(s)e^{s\tau} \neq \gamma \text{ for } \forall\ s \in \mathbb{C}_+\}$.

This lemma provides a graphical criterion for marginal stability of $\mathcal{H}_\bullet(s)$. In particular, the gain and phase monotonicity of $\phi(s)e^{s\tau}$ allows us to show that the frequency ϖ that satisfies both of the conditions (i) and (ii) in Proposition 1 is uniquely determined as follows.

Proposition 2. The frequency ϖ that satisfies both (i) and (ii) in Proposition 1 is unique. In particular, ϖ is given by the minimum positive solution of $\phi(j\varpi)e^{j\varpi\tau} = \lambda_1$.

The proof can be found in [16]. This proposition means that ϖ is given by the intersection at π/N that is the closest to the origin as illustrated in Fig. 3. Thus, geometric consideration allows us to analytically derive the frequency of oscillations in terms of the biological parameters (see [16] for the proof).

Theorem 1. *Consider the cyclic gene regulatory network with time delay modeled by (1). Then, the frequency ϖ of the oscillatory protein concentrations is expected to be the minimum positive solution of*

$$\varpi = \left(-\frac{\cot(\frac{\pi}{N} - \varpi\tau)}{Q} + \sqrt{\frac{\cot^2(\frac{\pi}{N} - \varpi\tau)}{Q^2} + 1} \right) \frac{1}{T_G}, \quad (20)$$

where $T_G := \sqrt{T_a T_b}$.

The frequency of oscillations is analytically predicted in this theorem. Thus, we can easily interpret the relation between the parameters and the frequency, since (20) is written only in terms of the given biochemical constants, namely N, Q, τ and T_G. Biological meanings of the above result will be seen in Section V-A, .

Remark 2. The minimum positive solution of (20) can be efficiently computed by the bisection search for $\varpi \in [0, \frac{\pi}{N\tau}]$,

because it is easily seen that the right-hand side of (20) monotonically decreases with respect to $\varpi \in [0, \frac{\pi}{N\tau}]$, and the minimum positive solution of (20) exists in this region.

C. Estimation of phase

The phase of the oscillations is given as the corresponding eigenvector to the eigenvalue $\phi(j\varpi)e^{j\varpi\tau}$ of $U^{-1}(j\varpi)K_1$. Thus, the goal of this section is to compute the eigenvector $\boldsymbol{y}$ in (18), and predict the phase of the oscillations. It should be noted that $U(j\varpi)$ becomes a complex valued constant matrix once ϖ is determined by (20). Thus, we hereafter write $U(j\varpi)$ as U.

Let $D := \mathrm{diag}(d_1, d_2, \cdots, d_N) \in \mathbb{C}^{N\times N}$ be defined by

$$d_i := \begin{cases} (-1)^{i-1}\dfrac{\prod_{k=1}^{i}\xi_k^* e^{j\varpi(\tau-\tau_k)}}{\left|\prod_{k=1}^{N}\xi_k^*\right|^{\frac{i-1}{N}}} & (\text{if } N \text{ is odd}) \\ \dfrac{\prod_{k=1}^{i}\xi_k^* e^{j\varpi(\tau-\tau_k)}}{\left|\prod_{k=1}^{N}\xi_k^*\right|^{\frac{i-1}{N}}} e^{-j\frac{i}{N}\pi} & (\text{if } N \text{ is even}) \end{cases} \tag{21}$$

with $\xi_i^* := \xi_i(x_{i-1}^*, y_{i-1}^*)$. It can be seen that $D^{-1}(U^{-1}K)D$ becomes the circulant matrix V, where

$$V := \begin{cases} |\prod_{k=1}^{N}\xi_k^*|^{\frac{1}{N}}\,\mathrm{cyc}(-1,-1,\cdots,-1) & (\text{if } N \text{ is odd}) \\ |\prod_{k=1}^{N}\xi_k^*|^{\frac{1}{N}}\,\mathrm{cyc}(e^{\frac{j\pi}{N}}, e^{\frac{j\pi}{N}}, \cdots, e^{\frac{j\pi}{N}}) & (\text{if } N \text{ is even}). \end{cases}$$

Then, it follows that the eigenvector $\boldsymbol{v}$ of V associated with the eigenvalue $\phi(j\varpi)e^{j\varpi\tau}$ is given as $\boldsymbol{v} := [v_1, v_2, \cdots, v_N]^T$ with

$$v_i := \begin{cases} (-1)^i e^{-j\frac{i-1}{N}\pi} & (\text{if } N \text{ is odd}) \\ 1 & (\text{if } N \text{ is even}). \end{cases} \tag{22}$$

Therefore, $\boldsymbol{y}$ is obtained by $\boldsymbol{y} = D\boldsymbol{v}$. Finally, computing $\varphi_i = \hat{\varphi}_i + \varpi\tau_{p_i}$ $(i = 1, 2, \cdots, N)$ yields the following analytic estimate of the phase.

Theorem 2. *Consider the cyclic gene regulatory network with time delay modeled by (1). The phase shift $(\varphi_{i+1} - \varphi_i)$ between the $(i+1)$-th and the i-th protein is expected as*

$$\varphi_{i+1} - \varphi_i = \left(Z - \frac{1}{N}\right)\pi - \varpi\Delta\tau_i. \tag{23}$$

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

$$\Delta\tau_i := (\tau_{r_{i+1}} + \tau_{p_i}) - \tau, \tag{24}$$

$$Z := \begin{cases} 1 & \text{if } f_{i+1}(\cdot) \text{ is a decreasing function} \\ 0 & \text{if } f_{i+1}(\cdot) \text{ is an increasing function} \end{cases}, \tag{25}$$

and ϖ is given by Theorem 1.

This theorem predicts the phase difference between protein species. A difference from the non-delay case [9] is that the phase depends on the frequency ϖ, and thus it also depends on Q, T_G and τ. It should be noted that $\Delta\tau_i$ is the displacement of the time delay in the i-th gene from the average delay τ.

The bias $\boldsymbol{x}$ and the amplitude $|\boldsymbol{y}|$ of the oscillations can also be derived from the bias and harmonic balanced equations. The details are presented in [16].

V. Interpretation of the main result

In this section, we provide some insights on how the time delay in transcription and translation process affects the oscillation profiles based on the theorems derived in the previous section. Illustrative numerical simulations are conducted to confirm the insights.

A. Effects of the time delay

We first consider the relation between the frequency and the time delay. Let $\hat{\varpi}$ denote a normalized frequency $\hat{\varpi} := \varpi T_G$ with T_G in Theorem 1. Then, (20) can be written as

$$\hat{\varpi} = \left(-\frac{\cot(\frac{\pi}{N} - \hat{\varpi}\hat{\tau})}{Q} + \sqrt{\frac{\cot^2(\frac{\pi}{N} - \hat{\varpi}\hat{\tau})}{Q^2} + 1}\right), \tag{26}$$

where $\hat{\tau}$ is the dimensionless parameter defined by (7). This implies that time can be normalized by $T_G = 1$ without loss of generality, and the frequency of the oscillations is essentially determined by (26). Thus, the dimensionless quantity $\hat{\varpi}$ essentially captures the quantitative relation of the frequency and the biological parameters.

We see from (26) that the frequency depends only on $\hat{\tau}$, N and Q (see Table I for biological meanings). In particular,

(A) the average time delay over all genes, $\hat{\tau}$, is a dominant factor to determine the frequency rather than the individual time delay of each gene.

Moreover, we see that the frequency ϖ becomes large as (i) the average time delay of transcription and translation process (τ) decreases, and (ii) the number of genes (N) decreases, and (iii) the mRNA and protein degradation time gets close to each other. In addition, we see that the frequency is bounded from above by $\varpi \le 1/T_G$, since $0 < \hat{\varpi} \le 1$ holds, and $\varpi = 1/T_G$ is achieved when $N = 2$ and $\hat{\tau} = 0$.

Next, we focus on the phase of the oscillations. The effect of the time delay appears in the last term of (23), $\varpi\Delta\tau_i$. Note that (23) exactly coincides with Theorem 2 in [9] when $\Delta\tau_i = 0$ $(i = 1, 2, \cdots, N)$.

Let $\Delta\hat{\tau}_i$ be defined by $\Delta\hat{\tau}_i := (\hat{\tau}_{r_{i+1}} + \hat{\tau}_{p_i}) - \hat{\tau}$ $(i = 1, 2, \cdots, N)$ with $\hat{\tau}_{r_i} := \tau_{r_i}/T_G$ and $\hat{\tau}_{r_i} := \tau_{r_i}/T_G$. It follows that $\varphi_{i+1} - \varphi_i = (Z - 1/N)\,\pi - \hat{\varpi}\Delta\hat{\tau}_i$. This means that

(B) the difference of the individual time delay from the average $\hat{\tau}$ affects the phase of oscillations.

Therefore, we see from the above observations (A) and (B) that the time delay allows the gene regulatory network to tune the phase of the oscillations without changing the frequency, which would be impossible when there is no time delay. In other words, the frequency and the phase can be somewhat independently regulated by the time delay. This will be confirmed in the following numerical examples.

B. Numerical examples

Consider the gene regulatory network, where $N = 6$ genes are involved as depicted in Fig. 4 (Left). Suppose the rate constants are given by $a_1 = a_2 = \cdots = a_6 = 3.0, b_1 = b_2 = \cdots = b_6 = 1.0$, $c_1 = c_3 = c_4 = c_6 = 3.2, c_2 = 2.8, c_5 =$

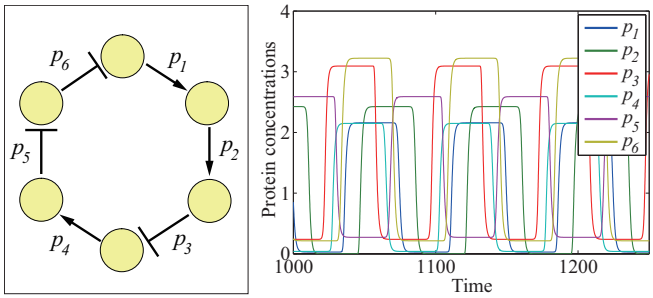

Fig. 4. Numerical simulation result in Section V-B. (Left) schematic diagram of the cyclic gene regulatory network with $N = 6$. (Right) oscillatory protein concentrations in Example 2.

3.7, $\beta_1 = \beta_4 = \beta_5 = 2.1, \beta_2 = \beta_3 = 2.9, \beta_6 = 3.1$. The nonlinear functions are set as Hill functions, *i.e.*, $f_1(\cdot) = f_4(\cdot) = f_6(\cdot) = F_R(\cdot)$ and $f_2(\cdot) = f_3(\cdot) = f_5(\cdot) = F_A(\cdot)$, where $F_R(p) := 1/(1+p^\nu)$ and $F_A(p) := p^\nu/(1+p^\nu)$ with the Hill coefficient $\nu = 2.8$. Then, the constants Q and T_G can be obtained as $Q = 0.866$ and $T_G = 0.577$.

We compare the two networks both of which have the same parameters shown above, but different time delays. We then confirm the insight obtained in Section V-A.

Example 1. Let $\boldsymbol{\tau}_r = [3.5, 3.5, 3.5, 3.5, 3.5, 3.5]^T$ and $\boldsymbol{\tau}_p = [1.5, 1.5, 1.5, 1.5, 1.5, 1.5]^T$, where the i-th entries of $\boldsymbol{\tau}_r$ and $\boldsymbol{\tau}_p$ correspond to τ_{r_i} and τ_{p_i}, respectively. It is obvious that

$$\tau = 5.0, \quad \Delta\boldsymbol{\tau} = [0, 0, 0, 0, 0, 0]^T, \tag{27}$$

where the i-th entry of $\Delta\boldsymbol{\tau}$ represents $\Delta\tau_i$. Using Theorem 1 and 2, we obtain the estimation of the oscillation profiles as follows.

Estimated frequency ϖ [rad/s]	8.27×10^{-2}
Actual frequency (simulation)[rad/s]	8.87×10^{-2}

Protein	p_2	p_3	p_4	p_5	p_6
Estimated [deg]	330.0	120.0	90.0	240.0	30.0
Actual [deg]	331.2	117.3	88.4	239.7	30.1

Note that the phase of $p_1(t)$ is set to zero, *i.e.*, $\varphi_1 = 0$. We see that the estimated values approximate the actual one obtained by a numerical simulation of (1). More detailed description of this example can be found in [16].

Example 2. Let the time delays be defined as $\boldsymbol{\tau}_r = [0.5, 8.0, 6.0, 2.0, 0.5, 0.5]^T$ and $\boldsymbol{\tau}_p = [2.0, 4.0, 3.0, 1.0, 1.0, 1.5]^T$. Then,

$$\tau = 5.0, \quad \Delta\boldsymbol{\tau} = [5.0, 5.0, 0.0, -3.5, -3.5, -3.0]^T. \tag{28}$$

Note that the average time delay τ is equal to that in Example 1, but $\Delta\boldsymbol{\tau}$ is different. Therefore, it is expected from the observations (A) and (B) in the previous section that the phase pattern of the oscillations is different from Example 1, while the frequency is almost the same. This statement can be verified from the table shown below.

Estimated frequency ϖ [rad/s]	8.27×10^{-2}
Actual frequency (simulation)[rad/s]	8.46×10^{-2}

Protein	p_2	p_3	p_4	p_5	p_6
Estimated [deg]	306.3	72.6	42.6	209.2	15.8
Actual [deg]	318.1	86.0	53.5	216.9	20.6

The numerical simulation result of the protein time course is shown in Fig. 4 (Right).

The accuracy of the estimation depends on how much the actual oscillations satisfy the assumption (15). Some remarks on the estimation error is presented in [16].

VI. Conclusion

In this paper, we have considered oscillation profiles of protein levels in cyclic gene regulatory networks with time delay. Based on the harmonic balance method, frequency and phase of oscillations have been analytically obtained in terms of the biological parameters. Then, we have interpreted the analytic results and showed that the time delay plays a key role to tune the phase of oscillations without changing the frequency. These insights were confirmed with illustrative numerical simulations.

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