Comparing two models based on the transcriptional regulation by KaiC of cyanobacteria rhythm

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***Abstract*—Circadian clocks are self-sustained biological oscillators that can be entrained by environmental cues. Cyanobacteria are the simplest organisms known to exhibit circadian rhythms, which is the fundamental process of homeostasis adapting to daily environmental changes. The cyanobacterial clock gene products, KaiA, KaiB, and KaiC interact with each other, and regulate KaiC phosphorylation and kaiBC expression in a circadian fashion. The total phosphorylation level of KaiC oscillates with a circadian period. In this paper, based on two possible transcriptional regulations, we examined numerically two models, the Transcriptional Activation Model and the Transcriptional Repression Model to generate circadian oscillation of *kai* genes. These two models both reproduce experimental observed sustained circadian oscillations in constant dark(DD) and constant light(LL). Comparing phase shifts between DD and LL in these two model, the Transcriptional Activation Model is consistent with the experimental observations, suggesting that the Transcriptional Activation Model may reflect the essence of the actual mechanism of *kai* oscillator in cyanobacteria.**

I. Introduction

Many organisms have endogenous circadian clocks that coordinate physiological and behavioral rhythms and synchronize the organisms to daily environmental cycles [1]. Circadian rhythms usually continue to keep the cyclic behavior even under constantly retained conditions, in the absence of any time-cues from the environment. This is called free-run and is the primary evident showing that circadian rhythmicity is intrinsic and includes self-sustained oscillators. In some organisms, circadian rhythms have been proved to be based on cyclic oscillation in transcriptional level of responsible genes. Such genes, which are often called clock genes, form networks of transcriptional interactions including feedback loops to generate autonomous oscillatory dynamics.

The simplest one among organisms exhibiting circadian rhythms is the cyanobacteria, in which the gene cluster *kaiABC* and its product proteins KaiA, KaiB, and KaiC are essential for generating the rhythm [2]. The *kai* genes form a gene cluster, where *kaiB* and *kaiC* are co-transcribed as *kaiBC* mRNA. KaiC plays a central role which is an enzyme and catalyzes the phosphorylation and dephosphorylation of two of its own residues in an ordered pattern [3]. KaiA and KaiB work together to modulate the activity of KaiC in a phosphorylation-dependent manner, where KaiA enhances autophosphorylation of KaiC and KaiB inhibits this action of KaiA, resulting in stable oscillations in the amount of phosphorylated KaiC [4]–[7].

KaiC overexpression consistently reduces kaiBC promoter activity, so it has been considered that KaiC regulates *kaiBC* transcription negatively [2]. This self-repression of KaiC may give an idea that oscillation of *kai* gene expression can be understood by the negative feedback transcriptional regulation as the case in *Drosophila* [8]. However, in the case of *kai* oscillator, this presupposition is not consistent with some experimental facts. Such as the *kaiC* absence did not show increase of *kaiBC* expression, but show decrease of it [2]. In addition, KaiA overexpression showed different effects on *kaiBC* transcription depending on KaiC and the transcriptional regulation by KaiC also shows *kaiA* dependency [9]. KaiA and KaiC seem to regulate *kaiBC* transcription in an unknown cooperative way, which makes it difficult to predict the transcriptional regulation by KaiA and KaiC. Considering the fact that KaiA enhances KaiC phosphorylation, it is suggested that this cooperative regulation by KaiA and KaiC is realized by KaiC phosphorylation. Actually, it was observed that overexpression of the nonphosphorylatable KaiC mutant only transiently represses kaiBC transcription, which is different from the result of overexpression of wildtype KaiC. This result gives a proof of the existence of the phosphorylation-dependent switch by KaiC [10].

The transcriptional regulation of *kaiBC* is not fully understood yet, though experimental evidences suggest the autoregulation by KaiC, which may work as positive or negative regulator depending on the phosphorylation status. Hisako and his collaborator developed a mathematical model for the dynamics of cyanobacterial circadian rhythms focusing on the transcriptional regulation by KaiC and predicted the transcriptional regulation mechanism by KaiC [11]. They found two mechanisms of the transcriptional regulation. One is the Transcriptional Repression Model where KaiC represses transcription of the clock genes after phosphorylation, and the other is the Transcriptional Activation Model where KaiC induces transcription after phosphorylation. phosphorylation level of KaiC oscillates with a circadian period. In this paper, we will examine numerically these two models whether they can generate sustained circadian oscillation in DD and LL as experiment observed. At the same time, we will compare

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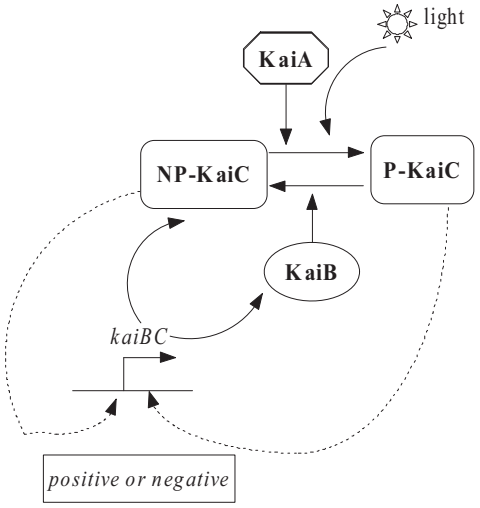

Fig. 1. Schematic diagram representing the model for the cyanobacteria circadian clock involving the process of *kaiBC* transcription and KaiC Phosphorylation. KaiB and KaiC are products of *kaiBC* translation. KaiA accelerates the phosphorylation of KaiC and KaiB accelerates the dephosphorylation. Transcriptional regulations of KaiC protein on *kaiBC* depend on its status. Phosphorylated KaiC and non-phosphorylated KaiC regulate *kaiBC* transcription positively or negatively.

the phase shifts between DD and LL in these two model numerically.

II. Description of the Mathematical Model

The model of the cyanobacteria circadian oscillator is schematized in Fig. 1. Considering the experimental results, phosphorylated KaiC(P-KaiC) and non-phosphorylated KaiC(NP-KaiC) may play different roles in the transcriptional regulation. We developed a model that describes interactions between the clock gene products; *kaiBC* mRNA, KaiA, KaiB, and KaiC in continuous time and states.

The following assumptions and rationale of the assumptions are invoked in developing the mathematical model given in Fig.1:

(1) *kaiBC* transcription is regulated by NP-KaiC and P-KaiC. The transcription rate of *kaiBC* is a function depending on the levels of NP-KaiC and P-KaiC.

(2) The NP-KaiC concentration increases with translation from *kaiBC*. The rate is assumed to be proportional to the level of *kaiBC*, and the rate constant is p.

(3) Phosphorylation of NP-KaiC increases P-KaiC, and dephosphorylation of P-KaiC increases NP-KaiC. The phosphorylation/dephosphorylation function depending on the concentrations of substrates NP-KaiC and P-KaiC. The concentrations of KaiA and KaiB are assumed to enhance and attenuate phosphorylation, respectively, and they are included in the phosphorylation/dephosphorylation function as well. The degradation rates of *kaiBC*, NPKaiC, and P-KaiC are assumed to be proportional to their concentrations, and the rate constants are δ_1, δ_2, and δ_3, respectively.

(4) It has been observed that the amount of KaiA in a single cell remains constant at a low level in the cytosol, and that the amount of KaiB is always kept proportional to that of KaiC [12]. We use a three-variable model to discribe the oscillatory behavior of *kaiBC* level and KaiC phosphorylation.

The dynamics of the model, schematized in Fig.1, is described by the following system of 3 differential equations:

$$\dot{X_1} = \frac{1}{1+\beta\exp[\alpha(k_1X_2 - k_2X_3)]} - \delta_1 X_1, \quad (1)$$

$$\dot{X_2} = pX_1 - \frac{K_1X_2}{K_2+X_2} + \frac{K_3(X_2+X_3)X_3}{K_4+X_3} - \delta_2 X_2, \quad (2)$$

$$\dot{X_3} = \frac{K_1X_2}{K_2+X_2} - \frac{K_3(X_2+X_3)X_3}{K_4+X_3} - \delta_3 X_3 \quad (3)$$

where X_1, X_2, and X_3 are the concentration of *kaiBC* mRNA, that of NP-KaiC and P-KaiC, respectively. k_1 and k_2 are both nonnegative.

The transcriptional regulation of phosphorylated and non-phosphorylated KaiC on *kaiBC* mRNA can be reflected by the value of α in Eq.1. If $\alpha > 0$, non-phosphorylated KaiC represses the transcription of *kaiBC* mRNA and phosphorylated KaiC induces the transcription of *kaiBC* mRNA, which corresponds to the Transcriptional Activation Model. Similarly, $\alpha < 0$ corresponds to the Transcriptional Repression Model. KaiA- and KaiB-mediated KaiC autophosphorylation are described by Michaelis-Menten function. K_1 indicates the maximum phosphorylation rate including KaiA activity. K_2 and K_4 are Michaelis constants. K_3 indicates the maximum dephosphorylation rate induced by KaiB activity [11].

Because parameter values of the model cannot be reliably known from experimental data, we choose their appropriate values based on previous models [11] with modifications so as to satisfy that the model must generate circadian oscillations with a period close to 24 h. The system of ordinary differential equations was solved numerically by using a 4th order Runge-Kutta algorithm. All simulations were implemented using Matlab.

III. Results

A. The model can simulate cyanobacteria circadian oscillation in DD

For a model of the circadian clock, we should first test whether its dynamics behavior is consistent with experimental observations. With parameter values in Fig.2 and Fig.3, our model can reproduce experimental observed sustained oscillations(see Fig.2 and Fig.3). In the Transcriptional Activation Model, the circadian period is about 25h, and 24.5h in the Transcriptional Repression Model in DD.

The time course of P-KaiC concentration are plotted in Fig.2 and Fig.3. It can be seen that the oscillation of P-KaiC concentration is acute and the amplitude is large in the Transcriptional Activation Model(Fig.2). Whereas the evolution of P-KaiC concentration is tardigrade and the amplitude is small, with larger values in the Transcriptional Repression Model(Fig.3). The reason making for this phenomenon may be that the phosphorylation/dephosphorylation function in these two models shows different evolution track

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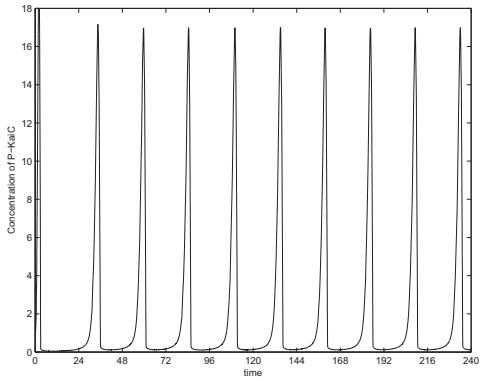

Fig. 2. Sustained circadian oscillations for the concentrations of P-KaiC in the Transcriptional Activation Model in DD. The result was obtained when $\beta = 148.4, \alpha = 4, k_1 = 1, k_2 = 1, \delta_1 = 0.1, p = 20, K_1 = 50, K_2 = 0.14, K_3 = 1.6, K_4 = 0.14, \delta_2 = 0.15, \delta_3 = 0.15$.

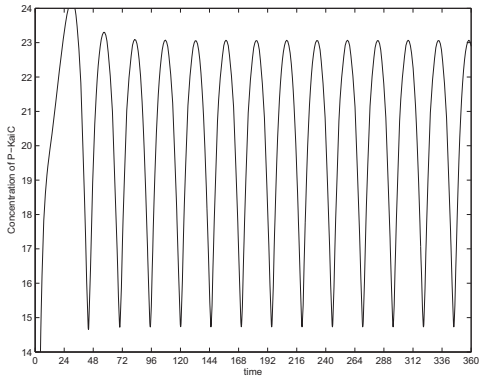

Fig. 3. Sustained circadian oscillations for the concentrations of P-KaiC in the Transcriptional Repression Model in DD. The result was obtained when $\beta = \exp(-75), \alpha = 5, k_1 = 0, k_2 = -1, \delta_1 = 0.1, p = 20, K_1 = 6, K_2 = 5, K_3 = 0.01, K_4 = 10, \delta_2 = 0.2, \delta_3 = 0.2$.

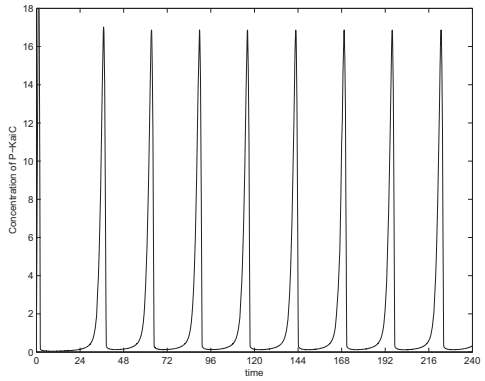

Fig. 4. Sustained circadian oscillations for the concentrations of P-KaiC in the Transcriptional Activation Model in LL. $L_{light} = 1$ and the other parameter values are the same as in Fig.2.

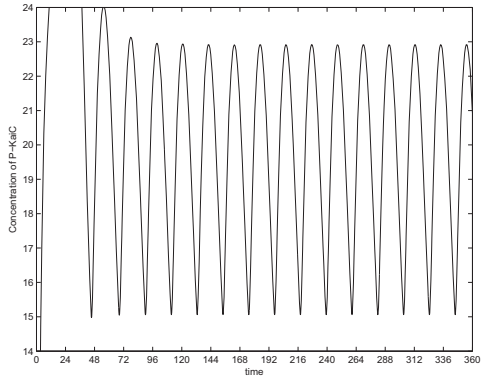

Fig. 5. Sustained circadian oscillations for the concentrations of P-KaiC in the Transcriptional Repression Model in LL. $L_{light} = 1$ and the other parameter values are the same as in Fig.3.

[11]. In the Transcriptional Activation Model, the phosphorylation/dephosphorylation function changes periodically, of which the amplitude is large and takes positive and negative values. In the Transcriptional Repression Model, the phosphorylation/dephosphorylation function also shows periodic oscillation, though the amplitude is small and the value is always positive.

B. Cyanobacteria circadian oscillation in LL

Cyanobacteria growing in the light primarily synthesize adenosine triphosphate (ATP) by photophosphorylating adenosine diphosphate (ADP) at the thylakoid membrane [13], and changes in culture illumination have been reported to affect the relative abundance of adenine nucleotides [14]–[16]. Michael *et.al* have tested incubating synchronized cultures in the dark under conditions sufficient to cause a phase shift in the circadian clock would result in changes in the concentrations of ATP and ADP. During the pulse of increased ADP, KaiC phosphorylation was decreased relative to that in the control reaction, similar to changes in KaiC phosphorylation in vivo when cells were subjected to a dark pulse [17]. The above experimental observations show that the light can increase KaiC phosphorylation rate. So we will simulate the effect of light by adding a term to the right-hand side of the differential equation for P-KaiC, i.e.,

$$\dot{X_3} = \frac{K_1 X_2}{K_2 + X_2} - \frac{K_3(X_2 + X_3)X_3}{K_4 + X_3} - \delta_3 X_3 + L_{light}, \quad (4)$$

where L_{light} represents the phosphorylation rate induced by light.

Our model also shows that the circadian rhythmicity also is kept in LL when L_{light} is maintained at a constant value ($L_{light} = 1$)(see Fig.4 and Fig.5), which is consistent with experimental observations [17].

There is a phase shift between the constant light and constant dark conditions both in the Transcriptional Activation Model and the Transcriptional Repression Model(see Fig.5 and Fig.6). Specially, there is a phase lag in DD in the Transcriptional Activation Model, and there is a phase advance in DD in the Transcriptional Repression Model. Considering the experimental observations in [17], the phase shift in the Transcriptional Activation Model is consistent with the experimental observations.

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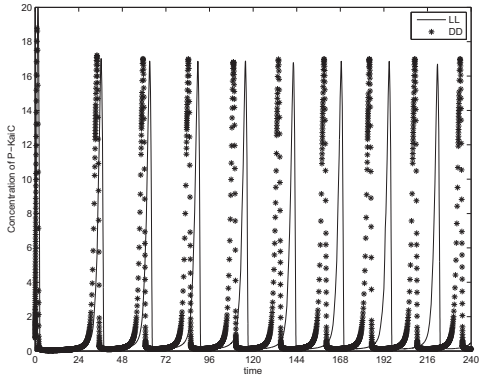

Fig. 6. Phase shift in the circadian clock in response to darkness for the concentrations of P-KaiC in the Transcriptional Activation Model.

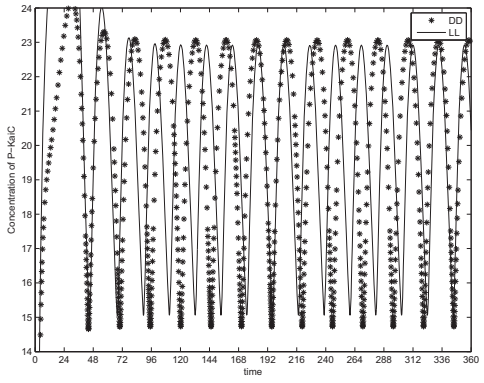

Fig. 7. Phase shift in the circadian clock in response to darkness for the concentrations of P-KaiC in the Transcriptional Repression Model.

IV. Conclusion and discussion

In this study, we performed the comprehensive study of current information on *kai* genes using the simple mathematical model focusing on the transcriptional regulation. Based on two possible transcriptional regulations [11], we examined numerically two models, the Transcriptional Activation Model and the Transcriptional Repression Model to generate circadian oscillation of *kai* genes. These two models both reproduce experimental observed sustained circadian oscillations in DD and LL. Comparing phase shifts between the constant light and constant dark conditions in these two model, the Transcriptional Activation Model is consistent with the experimental observations, suggesting that the Transcriptional Activation Model may reflect the essence of the actual mechanism of *kai* oscillator in cyanobacteria. Investigation using the mathematical method will provide insight into the circadian mechanism that, because of their complexity, cannot be comprehended by sheer intuition alone.

Acknowledgements

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