

Modelling circadian rhythms of protein KaiA, KaiB and KaiC interactions in cyanobacteria

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Abstract

Cyanobacteria are the simplest organisms known that exhibit circadian rhythms. The mechanism of circadian rhythm generation in cyanobacteria is different from eukaryotes. Based on the recent experiments about the interaction of KaiA, KaiB, and KaiC proteins with the generation of circadian rhythms *in vitro*, we developed a mathematical model to describe post-translational oscillations and the possible chemical reactions involved in the circadian clock mechanism of cyanobacteria. In this model, a series of differential equations, with linear kinetics for binding of proteins, Michaelis–Menten kinetics for enzymatic processes and a term including an explicit delay for dissociation of the KaiA/KaiB/phospho-KaiC complex, are proposed describing the dynamics of the chemistry. It is demonstrated that the mathematical system can lead to circadian oscillation within a range of parameter values.

Keywords: *Circadian rhythms, cyanobacteria, Kai, mathematical models, phosphorylation*

Introduction

Organisms have 24 h period circadian rhythms. From luminescence (Njus et al. 1981) and oxygen consumption (Karakashian & Schweiger 1976) in unicellular organisms to sleep in mammals, these biological activities are widely regulated by their circadian clock (Dunlap 1999). In most models, circadian clock is generated by the process that genes are transcribed and translated to proteins and subsequently, proteins control gene expression (Beersman 2005). It is a negative feedback loop (Hardin 2000; Roenneberg & Merrow 2003) and the process is called transcription-translation-derived oscillation (TTO) (Tomita et al. 2005).

The eukaryotic circadian clock is well studied. Several mathematical models were built to describe the molecular mechanism. In the early days, only two equations were used to describe the negative feedback loop of PER gene (Scheper et al. 1999). Currently, the models contain many equations for complex networks including many different genes, proteins and

other small molecules (Forger & Peskin 2003; Leloup & Goldbeter 2004). All of these are based on the TTO process.

Cyanobacteria are the simplest known organisms that exhibit circadian rhythms (Johnson et al. 1996). However, there are few mathematical models describing the cyanobacterial circadian clock to date. It may be due to the lack of experimental data in exploring the molecular mechanism of circadian rhythm generation in cyanobacteria. KaiA, KaiB and KaiC genes are primarily considered as essential components of cyanobacterial circadian clocks. KaiC represses KaiBC-promoter while KaiA enhances it (Ishiura et al. 1998). KaiC protein presents at auto-phosphorylation and auto-dephosphorylation (Xu et al. 2003; Kageyama et al. 2003). KaiC phosphorylation, together with KaiA and KaiB, is able to modify the level of phosphorylation (Kitayama et al. 2003). A recent experiment whereby KaiA, KaiB and KaiC proteins generate circadian rhythms *in vitro* demonstrates that the cyanobacterial circadian clock is originated from the interaction among the three proteins rather than the ordinary TTO process (Nakajima et al. 2005). The difference in molecular mechanism leads to different mathematical models. For example, the eukaryotic circadian clock models involve proteins and genes as well as the negative feedback loop among them. The cyanobacterial circadian clock models deal with only proteins without ordinary TTO feedback loops. In this paper, we develop a mathematical model for the oscillation generation based on the auto-phosphorylation and auto-dephosphorylation cycle (i.e. another type of feedback loop which is different from the TTO feedback loop formally) of KaiC and the change of relative molecules.

Materials and methods

KaiC post-translational modification

In the constant dark condition, cyanobacteria maintain circadian rhythms. However, the level of KaiBC mRNA promoter is almost undetectable. Thus the oscillation is not dominated by the activity of KaiC mRNA (Tomita et al. 2005). According to a KaiC post-translational modification model (Xu et al. 2003; Kageyama et al. 2003), KaiC phosphorylation is regulated by its own autokinase and auto-phosphatase activities. The decreasing level of KaiC phosphorylation results in the enhanced activity of auto-phosphorylation and the inhibited activity of auto-dephosphorylation. The existence of KaiA activates the effect of KaiC auto-phosphorylation, whereas the existence of KaiB attenuates KaiA's effect (Xu et al. 2003; Kageyama et al. 2003; Kitayama et al. 2003). The KaiC phosphorylation rhythm is accompanied by the formation of a series of KaiC complexes with KaiA and/or KaiB (Kitayama et al. 2003; Tomita et al. 2005). First, KaiC phosphorylation complexes are associated with KaiA. Consequently, the binding phosphorylated KaiC–KaiA interacts with KaiB. Finally, three proteins can be released from a transient KaiA–KaiB–KaiC complex. This results in the shift of KaiC from a phosphorylation-dominating state to a dephosphorylation-dominating state. KaiC post-translational modification can occur in the constant dark condition. The oscillation KaiC phosphorylation level dominates cyanobacterial circadian rhythms.

Figure 1 summarises the above-mentioned process of KaiC post-translational modification (Kitayama et al. 2003). KaiA, KaiB and KaiC proteins are all generated by gene transcription and translation *in vivo* (Nakajima et al. 2005). In our model, SasA genes and other molecules are not included because the SasA gene is not an essential component to generate the core oscillation. SasA and KaiC form an outer (secondary) loop.

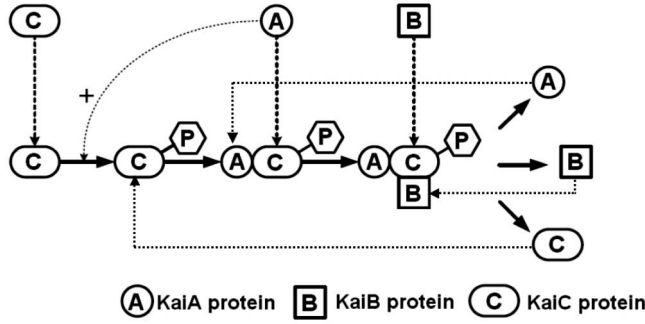


Figure 1. The process of KaiC post-translational modification. The real line denotes the route of interaction while the dashed denotes the proteins adding in. KaiC phosphorylation is regulated by its own autokinase and auto-phosphatase activities (the first term and the second term in Equations 3 and 4). The existence of KaiA activates the effect of KaiC auto-phosphorylation (the third term in Equations 3 and 4), whereas the existence of KaiB attenuates KaiA's effect (formally binds phosphorylated KaiC–KaiA and releases to increase the KaiC level). The KaiC phosphorylation rhythm is accompanied by the formation of a series of KaiC complexes with KaiA and/or KaiB. First, KaiC phosphorylation interacts with KaiA (the first term and second term in Equations 1 and 5, the fourth term and fifth term in Equation 4). Consequently, the binding phosphorylated KaiC–KaiA interacts with KaiB (the first term and second term in Equations 2 and 6, the third term and fourth term in Equation 5). Finally, three proteins are released from a transient KaiA–KaiB–KaiC complex. These results show the shift of KaiC from a phosphorylation-dominating state to a dephosphorylation-dominating state (the last term in Equations 1, 2, 3 and 6).

Mathematical models of protein KaiA, KaiB and KaiC interactions

Considering only the auto-phosphorylation and auto-dephosphorylation process of KaiC protein, we have,

$$\begin{aligned}\frac{dX_C}{dt} &= -k_p \frac{X_C}{\varphi_P + X_C} + k_{dp} \frac{X_{CP}}{\varphi_{CP} + X_{CP}} \\ \frac{dX_{CP}}{dt} &= k_p \frac{X_C}{\varphi_P + X_C} - k_{dp} \frac{X_{CP}}{\varphi_{CP} + X_{CP}},\end{aligned}$$

where X_C refers to the concentration of unphosphorylated KaiC, and X_{CP} refers to the concentration of phosphorylated KaiC. Solution of these two equations results in a steady state of KaiC phosphorylation without oscillation. If we introduce a time delay in either X_C or X_{CP} , there will be an oscillation between the concentrations of the two molecules. So these two equations are the core of our model, whereas many other molecules and relationships comprise the system and actually generate the time delay of the change of KaiC or KaiC phosphorylation protein levels. Therefore, we introduce other complex molecules that modify the levels of phosphorylated KaiC proteins and unphosphorylated KaiC proteins. Table I lists the parameters in our model. Note that the dimension of s^{-1} is changed to h^{-1} . The process in Figure 1 can then be described as:

$$\frac{dX_A}{dt} = -k_{fc1} X_A X_{CP} + k_{dc1} X_{ACP} + k_{dct} \frac{(X_{ABCP}^\tau)^n}{\theta_{ABCP}^n + (X_{ABCP}^\tau)^n} \quad (1)$$

$$\frac{dX_B}{dt} = -k_{fc2} X_B X_{ACP} + k_{dc2} X_{ABCP} + k_{dct} \frac{(X_{ABCP}^\tau)^n}{\theta_{ABCP}^n + (X_{ABCP}^\tau)^n} \quad (2)$$

Table I. Parameters in the mathematical models of protein KaiA, KaiB and KaiC interactions.

Parameter	Definition	Value (with the 22 h period)	Min (period) [†]	Max (period) [‡]	Range*
k_p	Rate constant for auto-dephosphorylation of KaiC protein	0.018 ($\mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$)	0.013 (24.0h)	0.1 (20.5h)	0.013–0.017
k_{dp}	Rate constant for auto-dephosphorylation of KaiC protein	0.005 ($\mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$)	0 (21.5h)	0.011 (24.0h)	0.008–0.011
k_{p1}	Rate constant for the formation of KaiA and phosphorylated KaiC	15 ($\mu\text{l} \cdot \mu\text{g}^{-1} \cdot \text{h}^{-1}$)	6.0 (22.5h)	>100 (21.8h)	6.0–12.0
k_{p2}	Rate constant for the formation of KaiA, KaiB, and phosphorylated KaiC	15 ($\mu\text{l} \cdot \mu\text{g}^{-1} \cdot \text{h}^{-1}$)	8.0 (22.4h)	>100 (29.4h)	8.0–13.0
k_{dc1}	Rate constant for the dissociation of KaiA and phosphorylated KaiC complex	0.1 (h^{-1})	0 (21.8h)	1.9 (22.5h)	0.5–1.9
k_{dc2}	Rate constant for the dissociation of KaiA, KaiB, and phosphorylated KaiC complex (dissociating to KaiA–KaiCP and KaiB)	0.1 (h^{-1})	0 (22.7h)	0.50 (20.6h)	0–0.02
k_{dca}	Rate constant for the dissociation of KaiA, KaiB, and phosphorylated KaiC complex (dissociating to KaiA, KaiB and KaiC)	0.022 ($\mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$)	0.018 (21.8h)	0.027 (22.4h)	0.024–0.027
k_{Ap}	Maximum rate for KaiC phosphorylation activated by KaiA	0.005 ($\mu\text{g} \cdot \mu\text{l}^{-1} \cdot \text{h}^{-1}$)	0 (22.2h)	0.08 (20.8h)	–
θ_{ABCP}	Michaelis constant for the dissociation of KaiA, KaiB, and phosphorylated KaiC complex (including KaiC dephosphorylation)	0.015 ($\mu\text{g} \cdot \mu\text{l}^{-1}$)	0.007 (22.6h)	0.030 (21.7h)	0.007–0.013
θ_A	Michaelis constant for KaiC phosphorylation activated by KaiA	0.05 ($\mu\text{g} \cdot \mu\text{l}^{-1}$)	0 (21.6h)	>1 (22.3h)	–
φ_P	Michaelis constant for KaiC auto-phosphorylation	0.015 ($\mu\text{g} \cdot \mu\text{l}^{-1}$)	0 (21.4h)	0.067 (23.7h)	0.022–0.067
φ_{CP}	Michaelis constant for KaiC auto-dephosphorylation	0.015 ($\mu\text{g} \cdot \mu\text{l}^{-1}$)	0 (22.1h)	>1 (21.1h)	–
τ	Time delay for the dissociation of KaiA, KaiB, and phosphorylated KaiC complex (including KaiC dephosphorylation)	9.5 (h)	2.5 (7.4h)	18.7 (41.7h)	12.6–18.7
n	Hill index of KaiC phosphorylation activated by KaiA	1	–	–	–
n	Hill index of the dissociation of KaiA, KaiB, and phosphorylated KaiC complex (including KaiC dephosphorylation)	3	–	–	–

[†]The minimum value of the parameters that can generate periodic oscillation.[‡]The maximum value of the parameters that can generate periodic oscillation.

*The range of the parameters that can generate periodic oscillation.

$$\begin{aligned} \frac{dX_C}{dt} = & -k_p \frac{X_C}{\varphi_P + X_C} + k_{dp} \frac{X_{CP}}{\varphi_{CP} + X_{CP}} - k_{Ap} \frac{X_A^m}{\theta_A^m + X_A^m} \\ & + k_{dct} \frac{(X_{ABCP}^\tau)^n}{\theta_{ABCP}^n + (X_{ABCP}^\tau)^n} \end{aligned} \quad (3)$$

$$\begin{aligned} \frac{dX_{CP}}{dt} = & k_p \frac{X_C}{\varphi_P + X_C} - k_{dp} \frac{X_{CP}}{\varphi_{CP} + X_{CP}} + k_{Ap} \frac{X_A^m}{\theta_A^m + X_A^m} \\ & - k_{fc1} X_A X_{CP} + k_{dc1} X_{ACP} \end{aligned} \quad (4)$$

$$\frac{dX_{ACP}}{dt} = k_{fc1} X_A X_{CP} - k_{dc1} X_{ACP} - k_{fc2} X_B X_{ACP} + k_{dc2} X_{ABCP} \quad (5)$$

$$\frac{dX_{ABCP}}{dt} = k_{fc2} X_B X_{ACP} - k_{dc2} X_{ABCP} - k_{dct} \frac{(X_{ABCP}^\tau)^n}{\theta_{ABCP}^n + (X_{ABCP}^\tau)^n} \quad (6)$$

$$* X_{ABCP}^\tau \stackrel{def}{=} X_{ABCP}(t - \tau).$$

In the above equations, X_A , X_B and X_C represent the concentrations of separate KaiA, KaiB and KaiC proteins, respectively. X_{CP} denotes the concentration of phosphorylated KaiC, X_{ACP} denotes the concentration of KaiA–KaiC complex, and X_{ABCP} denotes the concentration of KaiA–KaiB–KaiC complex. Due to the generation and release of KaiA–KaiB–KaiC complexes, this is a non-linear processor with a delay. Since more details of the reaction have not been available until now, we preferred to simplify the process.

The initial concentrations of KaiA, KaiB and KaiC, $0.05 \mu\text{g}/\mu\text{l}$, $0.05 \mu\text{g}/\mu\text{l}$ and $0.20 \mu\text{g}/\mu\text{l}$, respectively, were obtained from recent experiments (Tomita et al. 2005; Nakajima et al. 2005). These concentrations were estimated from the last test (Nakajima et al. 2005) and to be considered since they are the true quantity *in vivo* (Tomita et al. 2005), were used to validate our model. The order of the rate constants for KaiC protein phosphorylation and dephosphorylation are estimated from the experimental data, $10^{-3} \sim 10^{-4} \text{ s}^{-1}$ (Nakajima et al. 2005). In the experiment (Tomita et al. 2005), the performance of KaiC auto-phosphorylation is investigated in the presence or the absence of KaiA, so we can estimate the order of other parameters for KaiA-activated KaiC auto-phosphorylation by the change rate of protein concentrations.

We solve these equations with a Matlab function `dde23`. The `dde23` tracks discontinuities and integrates with the explicit Runge-Kutta (2,3) pair. It uses iteration to take steps longer than the lags.

Results and discussion

Periodic oscillation

The ratios of phosphorylated and unphosphorylated KaiC protein to KaiC protein produce periodic oscillations with the reverse phase. Figure 2 illustrates the time dependent concentration profiles of proteins for the system with steady oscillation. According to the experimental data reported by Nakajima et al. (2005), the period of the oscillations is about 22 h, which is verified by our model (see also Table I). We can simply change the period from 22 h to 24 h by adjusting some parameters (for example τ).

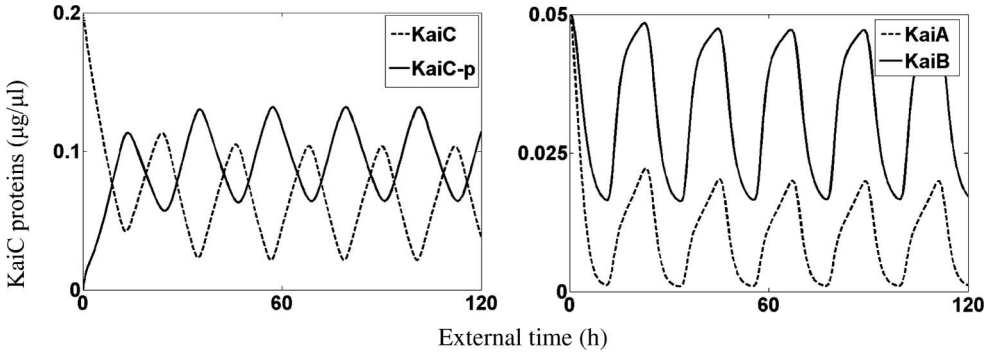


Figure 2. The concentrations of free proteins (KaiA, KaiB, KaiC proteins and KaiC phosphorylation) as a function of time.

Parameters for the model

Table I lists the range of each parameter for producing sustained oscillations in our model. For example, parameter k_p ranges from 0.013 to 0.1. In the range between 0.013 and 0.017, the calculated concentration of specific molecules (i.e. KaiA–KaiB–KaiC complex) is close to zero for all probabilities in the Matlab calculation. When $k_p > 0.1$, the oscillation is too faint to be detected. It can be considered as the steady state. Figure 3 shows the effect of various parameters (except for parameters m and n) on the oscillating periods of the phosphorylated and the unphosphorylated KaiC proteins.

We notice that parameter τ affects the oscillation period more dramatically than others such as k_p and k_{fc2} . Intuitively, it may be explained the fact that our model is based on the variation of binding and releasing molecules in the system. This is different from the model of eukaryotic circadian clock, which is based on the negative loops of mRNAs and corresponding proteins. In the early mathematical model of eukaryotic circadian clock, oscillation was generated by introducing a time delay (Scheper et al. 1999). The production and degradation rate constants of protein and mRNA are much more effective for the period. In the late models, the time delay has been replaced by a certain detailed molecular interactive process (Forger & Peskin 2003; Leloup & Goldbeter 2004), which affects the oscillation period (Leloup & Goldbeter 2004). In our model, the degradation of the KaiA–KaiB–KaiC phosphorylation complex is simplified as a non-linear process with a time delay between the KaiC auto-phosphorylation and auto-dephosphorylation. The simulation data agrees with the experimental results (Nakajima et al. 2005), indicating that the simplification is rational.

It is difficult to determine the ranges for parameters m and n . In fact, the effect of parameter m on oscillating period is not significant. As shown in Figure 4A, the oscillation is robust for a wide range of m ($m = 1, 3, 9$). The n value indicates the number of molecules in the process. This is reflected by the following term, $k_{dct} \frac{(X_{ABCP}^{\tau_1})^n}{\theta_{ABCP}^n + (X_{ABCP}^{\tau_1})^n}$, in Equations 1–6. When $n = 1$, the system cannot oscillate. When $n = 2$, the oscillation can hardly be detected. When $n > 3$, the system can oscillate robustly. The period of oscillation changes little even if $n = 9$ (21.6 h), however, the extent of the oscillation increases. In addition, the calculated concentration of specific molecules can be less than zero for very high n . Figure 4B shows the time-dependent concentration oscillation of phosphorylated KaiC protein with respect to different n values.

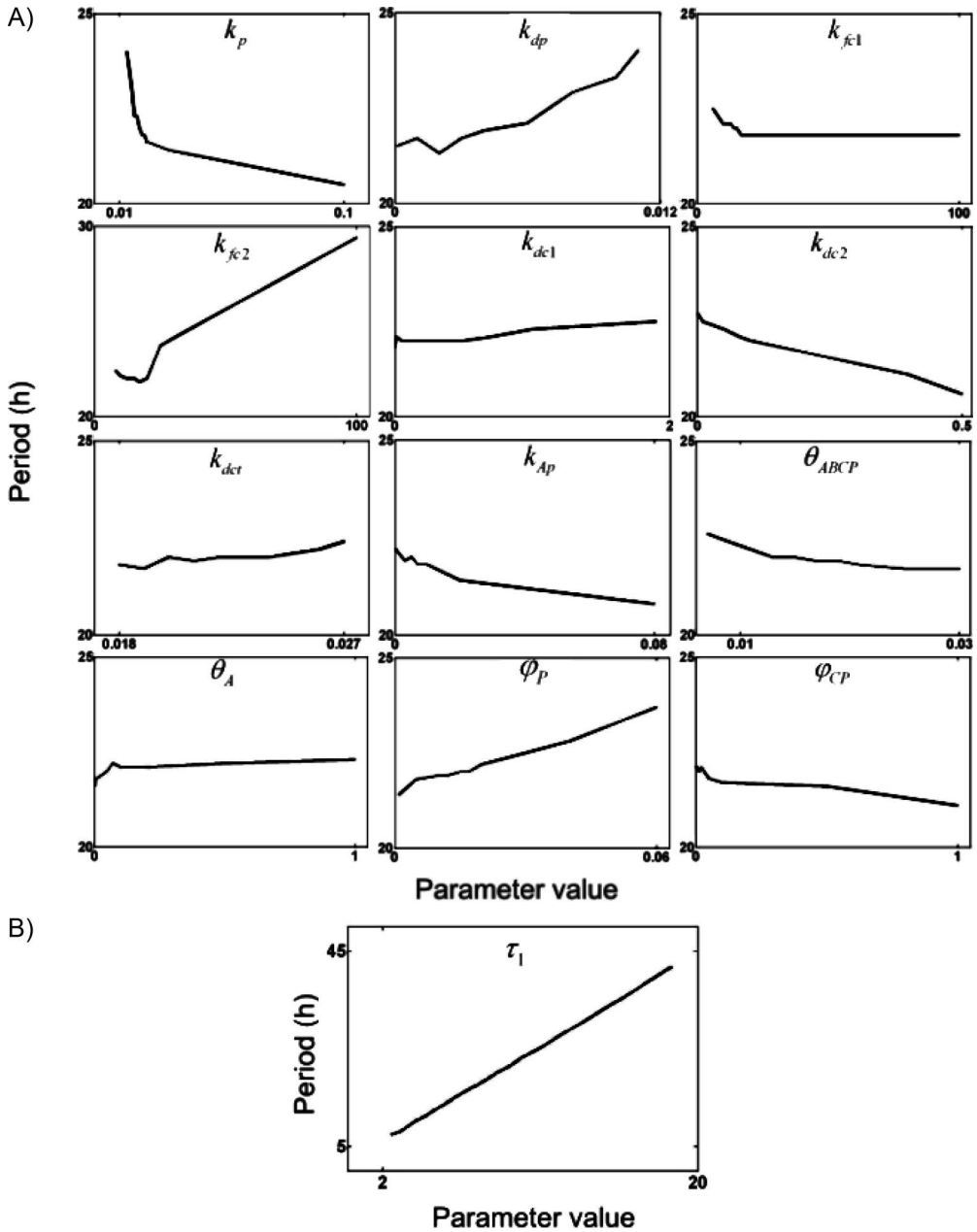


Figure 3. The variation of oscillation periods of phosphorylated and unphosphorylated KaiC proteins versus different parameters.

The effect of temperature compensation on oscillation period was investigated in the experiments of Tomita et al. (2005) and Nakajima et al. (2005). However, when the temperature rises from 25°C to 37°C, the period descends from 22 h to 21 h. This phenomenon can also be seen in our model. With increasing temperature, the interaction

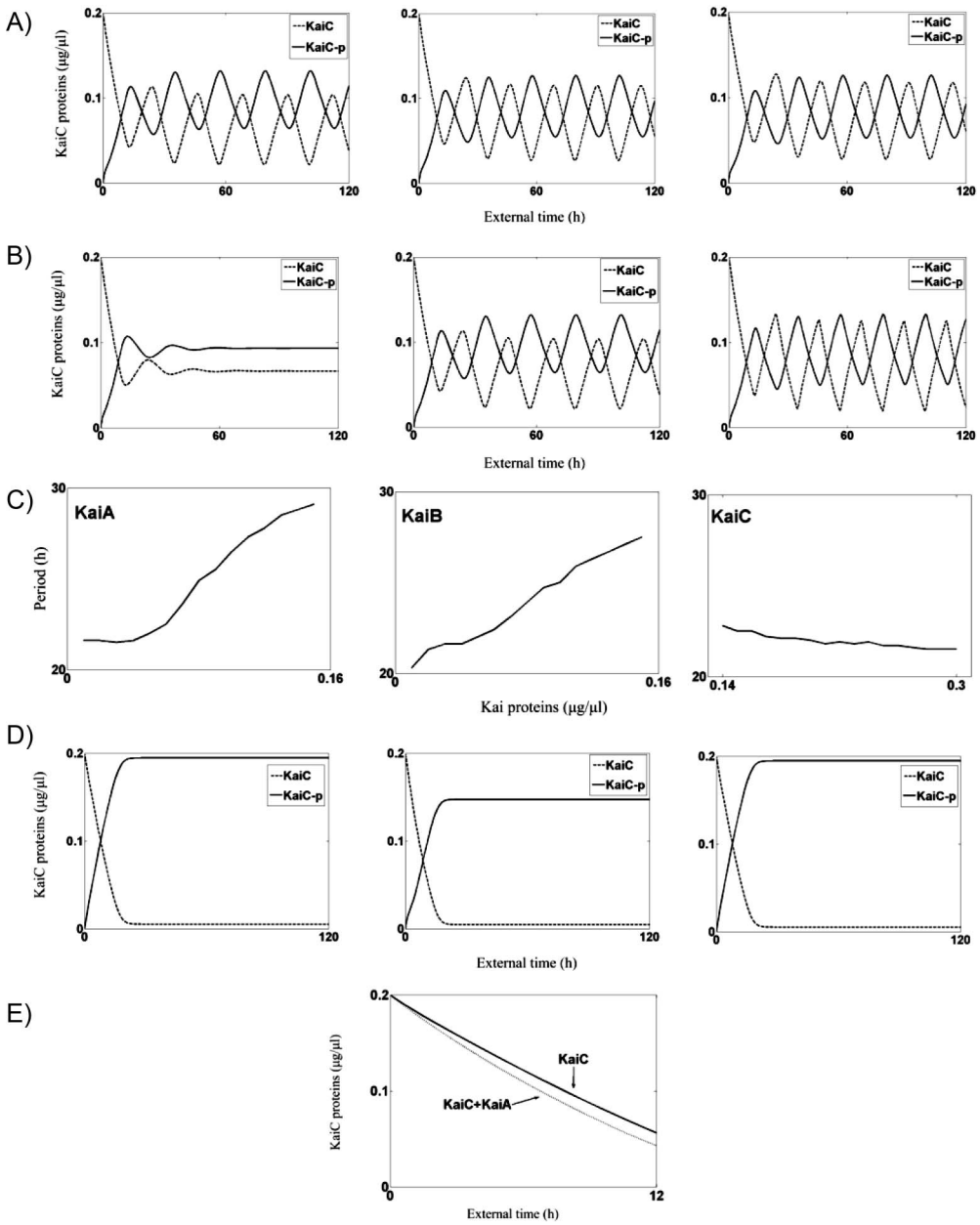


Figure 4. The oscillation profiles for KaiC protein and phosphorylated KaiC (A: $m = 1, 3, 9$; B: $n = 1, 3, 9$). (C) The effect of initial concentration on the period of phosphorylated and unphosphorylated KaiC protein. (D) The curve of concentrations of phosphorylated and unphosphorylated KaiC protein, when we remove KaiA, KaiB, KaiA and KaiB, respectively. Axis- X denotes the time, and axis- Y denotes the concentrations. (E) The concentration profile of phosphorylated KaiC protein in the duration of 0–12 h after removing KaiA and both KaiA and KaiB, respectively.

between molecules enhances. This leads to a fast release of the KaiA–KaiB–KaiC phosphorylation complex and a short time delay between KaiC auto-phosphorylation and auto-dephosphorylation. According to Equations 1–6, the decreasing τ value results in a short period.

Initial protein concentrations

According to the experiment reported by Nakajima et al. (2005), we initially set the measured concentrations of KaiA, KaiB and KaiC proteins to fit the 22 h period. When the initial concentrations are changed, the period also changes. From the viewpoint of stochastic, the lower concentrations denote the smaller number of molecules and therefore less chance of collision. So the oscillation period is sensitive to the initial concentrations of proteins.

Figure 4C shows the oscillation period as a function of initial concentrations of individual proteins. For KaiA protein, the oscillation cannot be detected when its concentration is less than 0.01. The period is slightly concentration dependent within the range 0.01–0.05. While in the range 0.05–0.20, the period increases with increasing initial concentrations. The oscillation disappears as the initial concentration is more than 0.20. Similarly for KaiB protein, the oscillation cannot be detected when its concentration is less than 0.01. The period is slightly concentration dependent within the range 0.01–0.05. Between 0.05 and 0.20, the period increases with increasing initial concentrations. However, the oscillation remains, as the initial concentration is more than 0.20. As for the KaiC protein, the system cannot oscillate when its initial concentration is less than 0.14. Between 0.14 and 0.17, the calculated concentration of specific molecules can be less than zero. The system oscillates robustly when the initial concentration of KaiC is more than 0.17. Interestingly, the period decreases slightly with increasing concentration. As such, the period of oscillation can be adjusted from the experimental values, 22 h, to the eukaryotic circadian clock, 24 h through modifying the initial concentration.

Obviously, we can set the initial concentrations of one protein to 0. It is equal to remove such type of protein in the system. Figure 4D shows the time dependent concentration profiles of phosphorylated and unphosphorylated KaiC proteins without KaiA, KaiB, or KaiA and KaiB, respectively. As expected, the oscillation of KaiC phosphorylation does not happen due to the lack of KaiA–KaiB–KaiC complex in such a system. The effect of removing KaiA is similar as the effect of removing both KaiA and KaiB proteins because the formation of the KaiA–KaiC complex is a prerequisite for KaiB binding with the complex. If KaiA does not exist in the system, KaiB will not affect KaiC phosphorylation (Xu et al. 2003; Kageyama et al. 2003; Kitayama et al. 2003; Iwasaki & Kondo 2004). In the case of removing KaiB from the system, the concentration of phosphorylated KaiC protein is lower than the concentration of removing KaiA since a part of the phosphorylated KaiC protein binds with KaiA. Moreover, KaiA can activate KaiC auto-phosphorylation. Figure 4E shows the concentration profile of phosphorylated KaiC protein during 0–12 h. The speed of KaiC auto-phosphorylation decreases when KaiA is removed. Thus, our model specifies the process of KaiA, KaiB, and KaiC protein interactions, which illustrates the existence of circadian rhythm in cyanobacteria even without the aid of gene activity.

Our mathematical model of the cyanobacterial circadian clock remains time independent of the transcription and the translation processes. It is different from other models of eukaryotes, such as *Drosophila* and mammalia (Scheper et al. 1999; Forger & Peskin 2003; Leloup & Goldbeter 2004), which are derived from the negative feedback loops between mRNAs and corresponding proteins. In this model, a series of differential equations, with linear kinetics for binding of proteins, Michaelis–Menten kinetics for enzymatic processes and a term including an explicit delay for the dissociation of the KaiA/KaiB/phospho-KaiC complex, are proposed describing the dynamics of the chemistry. It is demonstrated that the mathematical system can lead to circadian oscillation within a range of parameter values. Among them we show that the parameter τ_1 , which represents the delay, evidently impacts the circadian rhythms.

Whereas the change of three key parameters, the initial concentrations for protein KaiA and protein KaiB, especially for protein KaiC, slightly modifies the period and the extent of oscillations, such performance coincides with the experiments (Tomita et al. 2005; Nakajima et al. 2005) since the initial concentrations of KaiA, KaiB, and KaiC proteins are fixed in each test. Besides, additional experiment data that include different initial concentrations with the period are still desirable for evaluating and improving our model.

In vivo, the concentrations of KaiA, KaiB, and KaiC proteins are regulated by corresponding genes. Genes and proteins interaction can form robust negative feedback loops (Ishiura et al. 1998). Accordingly, in the constant light condition, the transcription-translation-derived oscillation of these three proteins in cyanobacteria will occur just like the TTO in eukaryotic. And what roles do mKaiA and mKaiBC (promoter) play in the circadian clock system? If we simplify the system model in the constant light condition, we suppose that there is a time when the genes are active and inactive instantaneously. After a time, the concentrations of three proteins are at the initial concentrations, and if the values of the concentrations are not properly calculated, the period of the circadian clock will change. Before that time, the interaction between promoters and proteins can form a robust negative feedback loop, a process that ensures that the values of the initial concentrations are maintained constant when the condition is changed. Thus, the role of mKaiA and mKaiBC is supposed to make enough “materials” for the process of KaiA, KaiB and KaiC protein interaction in the constant dark condition, and ensure that the quantity of the “materials” is steady.

Conclusions

In summary, based on the recent experiments showing an *in vitro* rhythm of KaiC protein phosphorylation, this work presents a mathematical model describing the post-translational oscillations and the possible chemical reactions involved in the circadian clock mechanism of cyanobacteria. Further experimental research is required to explore the relationship among oscillations of protein interactions and between TTO and oscillations.

Acknowledgements

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