Entrainment mechanism of the cyanobacterial circadian clock induced by oxidized quinone*

Ying Li(李莹)†, Guang-Kun Zhang(张广鹍), and Zi-Gen Song(宋自根)

College of Information Technology, Shanghai Ocean University, Shanghai 201306, *China*

(Received 4 June 2020; revised manuscript received 2 July 2020; accepted manuscript online 15 July 2020)

The circadian clock is a self-sustained biological oscillator which can be entrained by environmental signals. The cyanobacteria circadian clock is the simplest one, which is composed of the proteins KaiA, KaiB and KaiC. The phosphorylation/dephosphorylation state of KaiC exhibits a circadian oscillator. KaiA and KaiB activate KaiC phosphorylation and dephosphorylation respectively. CikA competing with KaiA for the same binding site on KaiB affects the phosphorylation state of KaiC. Quinone is a signaling molecule for entraining the cyanobacterial circadian clock which is oxidized at the onset of darkness and reduced at the onset of light, reflecting the environmental light–dark cycle. KaiA and CikA can sense external signals by detecting the oxidation state of quinone. However, the entrainment mechanism is far from clear. We develop an enhanced mathematical model including oxidized quinone sensed by KaiA and CikA, with which we present a detailed study on the entrainment of the cyanobacteria circadian clock induced by quinone signals. We find that KaiA and CikA sensing oxidized quinone pulse are related to phase advance and delay, respectively. The time of oxidized quinone pulse addition plays a key role in the phase shifts. The combination of KaiA and CikA is beneficial to the generation of entrainment, and the increase of signal intensity reduces the entrainment phase. This study provides a theoretical reference for biological research and helps us understand the dynamical mechanisms of cyanobacteria circadian clock.

Keywords: mathematical model, entrainment, cyanobacterial circadian clock, phase response curve

PACS: 87.18.Yt, 87.85.Tu, 87.18.Vf **DOI:** 10.1088/1674-1056/aba615

1. Introduction

Most organisms have developed endogenous circadian clocks to anticipate daily variations in their environment. Circadian clocks consist of a central oscillator together with input and output pathways.[1] The central oscillator generates the fundamental rhythm with a periodicity of approximately 24 h. The input pathway senses environmental signals, such as the daily light–dark cycle, and synchronizes the central oscillator to the external daily cycle by modifying the phase of the oscillation. The output pathway relays information from the oscillator to the downstream biochemical processes to control the physiology of the organism.[2]

A cyanobacterium is the simplest organism to have a circadian clock.[3] The central oscillator in cyanobacteria is known as the minimal self-sustained oscillator which consists of three proteins: KaiA, KaiB, and KaiC.[4] The phosphorylation state of KaiC oscillates with a period of nearly 24 h. The structure basis of the mechanism is well known.[5,6] KaiC can autophosphorylate and autodephosphorylate at both serine 431 (S431) and threonine 432 (T432).[7–10] KaiA actives the autophosphorylation of KaiC by binding to the A-loop, whereas KaiB weakens the activity of KaiA by sequestering KaiA from the A-loop,[11] which generates the oscillation of KaiC phosphorylation with a circadian period.[12] CikA, circadian input kinase A, has multiple effects on the *in vivo* clock.[13] CikA binds to KaiB at the same binding site that is used to sequester KaiA,[6] which results in an early release of KaiA from KaiB. This competitive interaction between CikA and KaiA changes the ability of KaiA to stimulate KaiC phosphorylation, which affects both the amplitude and the period of the circadian oscillation.[14]

In order to get entrainment, the circadian oscillator needs to adjust its phase with the light–dark cycle. When cyanobacteria receive an environmental light or dark signal, it is turned into biochemical signals, such as adenosine diphosphate (ADP) or quinone signals.[15,16] The oscillator itself can sense ADP signal directly and the input component, CikA, can sense quinone signals, which adjust the phases of the oscillator to achieve entrainment.[15,17,18] The signals usually affect the phosphatase activities of KaiC and result in phase advance or phase delay of phosphorylation, depending on the timing of adding signals, so that the endogenous rhythm can be properly reset.[18]

Quinone is a signaling molecule that can adjust the phase of the cyanobacterial circadian clock for the entrainment. Quinone is oxidized at the onset of darkness and reduced at the onset of light in cyanobacteria. Quinone entrainment experiments show that KaiA senses the redox state of quinone by binding quinone, which is involved in phase advances when oxidized quinone is added in the circadian clock mixture at CT 8.[18] CikA, as an input component, also senses the redox state of quinone by selectively binding to oxidized quinone.[17]

*Project supported by the National Natural Science Foundation of China (Grant No. 11672177).

†Corresponding author. E-mail: leeliying@163.com

CikA is involved in phase delays when oxidized quinone is added in the circadian clock mixture at CT 22.[18] Although many biological results have been found, the molecular mechanism is not fully understood.

Mathematical modeling has become a useful and powerful tool in investigating the regulatory mechanism of circadian clocks.[15,19–24] As for the entrainment mechanisms of the cyanobacterial circadian clock induced by ADP or direct light–dark cycle, there have some results from the perspectives of biological experiments and mathematical modeling.[15,25,26] However, to our knowledge, there have been only some biological findings about the entrainment induced by quinone.[17,18] So far, there is no research work on it from the mathematical angle. To explore the entrainment mechanism of cyanobacteria circadian clock induced by quinone, we bring forward an enhanced mathematical model. In this model, oxidized quinone is sensed by KaiA and CikA. We will analyze how quinone signals entrain the cyanobacteria circadian clock. The rest of this article is organized as follows. In Section 2, a mathematical model is introduced to describe the cyanobacterial circadian clock. The numerical results and analysis are presented in Section 3. Lastly, we show the conclusion and discussion in Section 4.

2. Mathematical model of cyanobacterial circadian clock

As we know the eukaryotic circadian clocks of mammal or *Drosophila* are based on the transcription-translation feedback loops, where the negative feedback loop is the key to produce sustained oscillators. However, the cyanobacterial oscillator is a post-translational oscillator comprised of KaiA, KaiB, and KaiC proteins. KaiC's daily rhythms of phosphorylation at residues S431 and T432 are key features of the timekeeping mechanism. KaiA actives the phosphorylation of KaiC and phosphorylated KaiC at the S431 site inhibits KaiA via KaiB. Thus a negative feedback loop is created, which plays a crucial role in oscillator generation.

Our mathematical model of phosphorylation and dephosphorylation cycles of KaiC with CikA and quinone signal is based on Rust's model of ordered multisite phosphorylation for the KaiABC oscillator.[24] The model is described by a three-dimensional system of ordinary differential equations which include the temporal dynamics of KaiC in four different forms: unphosphorylated, singly phosphorylated at the T432 site, singly phosphorylated at the S431 site, and doubly phosphorylated at both the S431 and T432 sites. This system is described as follows:

$$\begin{aligned}
\frac{\mathrm{d}T}{\mathrm{d}t} &= k_{\mathrm{UT}}U + k_{\mathrm{DT}}D - k_{\mathrm{TU}}T - k_{\mathrm{TD}}T,\\
\frac{\mathrm{d}S}{\mathrm{d}t} &= k_{\mathrm{US}}U + k_{\mathrm{DS}}D - k_{\mathrm{SU}}S - k_{\mathrm{SD}}S,\\
\frac{\mathrm{d}D}{\mathrm{d}t} &= k_{\mathrm{TD}}T + k_{\mathrm{SD}}D - k_{\mathrm{DT}}D - k_{\mathrm{DS}}D,\\
U &= [\mathrm{KaiC}] - T - S - D,\\
k_{XY}(S) &= k^0_{XY} + \frac{k^A_{XY}A(S)}{K_{1/2} + A(S)},
\end{aligned} \tag{1}$$

where T is the concentration of singly phosphorylated KaiC at the T432 site (T-KaiC), S is the concentration of singly phosphorylated KaiC at the S431 site (S-KaiC), D is the concentration of doubly phosphorylated KaiC at both the S431 and T432 sites (ST-KaiC), and U is the concentration of unphosphorylated KaiC (U-KaiC) which follows from conservation of total KaiC concentration ([KaiC]).

In Rust's monomer model, $A(S)$ represents the concentration of active KaiA, which is inhibited by S-KaiC via KaiB, so one has[24]

$$A(S) = \max\{0, [\mathrm{KaiA}] - 2mS\},$$

where $m = 1$. In this article, the addition of oxidized quinone indirectly affects KaiC's phosphorylation, which will be reflected in $A(S)$.

When KaiA senses quinone signals, KaiA binds oxidized quinone and loses its activity. KaiA mainly regulates the phosphorylation process of KaiC, so oxidized quinone affects the phosphorylation of KaiC via KaiA. During the phosphorylation process of KaiC, (i.e., in k_{UT}, k_{US}, k_{TD}, k_{SD}), we take $A(S) = \max\{0, [\mathrm{KaiA}] - 2S - Qu\}$ to reflect the effects of oxidized quinone on the phosphorylation of KaiC, where Qu is the concentration of oxidized quinone. During the dephosphorylation process of KaiC, the effect of oxidized quinone is not considered. That is to say, $A(S)$ is still $\max\{0, [\mathrm{KaiA}] - 2mS\}$ in k_{TU}, k_{SU}, k_{DT}, k_{DS} as that in Rust's model. In all, $A(S)$ is expressed as follows:

$$A(S) = \begin{cases} \max\{0, [\mathrm{KaiA}] - 2S - Qu\}, & \text{in } k_{\mathrm{UT}},\ k_{\mathrm{US}},\ k_{\mathrm{TD}},\ k_{\mathrm{SD}},\\ \max\{0, [\mathrm{KaiA}] - 2S\}, & \text{in } k_{\mathrm{TU}},\ k_{\mathrm{SU}},\ k_{\mathrm{DT}},\ k_{\mathrm{DS}}. \end{cases} \tag{2}$$

Similarly, when CikA senses quinone signals, oxidized quinone disrupts the binding interface of CikA and dissociates CikA from KaiB, which happens during the dephosphorylation phase.[6] Accordingly oxidized quinone affects KaiC's dephosphorylation via CikA. Considering CikA regulates the dephosphorylation process of KaiC by competitively binding to the same site of KaiB with KaiA, we also use $A(S)$ to reflect the effects of oxidized quinone on KaiC's dephosphorylation. During the

phosphorylation process of KaiC, the effect of oxidized quinone is not considered. Thus, $A(S)$ is expressed as follows:

$$A(S)=\begin{cases}\max\{0,[\text{KaiA}]-2S\}, & \text{in } k_{\text{UT}},\ k_{\text{US}},\ k_{\text{TD}},\ k_{\text{SD}},\\ \max\{0,[\text{KaiA}]-\dfrac{2S}{(1+[\text{CikA}])(1+Qu)}\}, & \text{in } k_{\text{TU}},\ k_{\text{SU}},\ k_{\text{DT}},\ k_{\text{DS}}.\end{cases} \tag{3}$$

We use the parameters estimated by Rust *et al.* from biological experiments,[24] namely $k^0_{\text{UT}}=0\ \text{h}^{-1}$, $k^0_{\text{TD}}=0\ \text{h}^{-1}$, $k^0_{\text{SD}}=0\ \text{h}^{-1}$, $k^0_{\text{US}}=0\ \text{h}^{-1}$, $k^0_{\text{TU}}=0.21\ \text{h}^{-1}$, $k^0_{\text{DT}}=0\ \text{h}^{-1}$, $k^0_{\text{DS}}=0.31\ \text{h}^{-1}$, $k^0_{\text{SU}}=0.11\ \text{h}^{-1}$, $k^A_{\text{UT}}=0.479077\ \text{h}^{-1}$, $k^A_{\text{TD}}=0.212923\ \text{h}^{-1}$, $k^A_{\text{SD}}=0.505692\ \text{h}^{-1}$, $k^A_{\text{US}}=0.0532308\ \text{h}^{-1}$, $k^A_{\text{TU}}=0.0798462\ \text{h}^{-1}$, $k^A_{\text{DT}}=0.1730000\ \text{h}^{-1}$, $k^A_{\text{DS}}=-0.319385\ \text{h}^{-1}$, $k^A_{\text{SU}}=-0.133077\ \text{h}^{-1}$, $K_{1/2}=0.43\ \mu\text{M}$, $[\text{KaiC}]=3.4\ \mu\text{M}$. We set $[\text{KaiA}]=1.07\ \mu\text{M}$ and $[\text{CikA}]=0.3\ \mu\text{M}$, with which the circadian clock has an inherent period of 21.1 h. In the following, using the above model, we will study the entrainment mechanism of cyanobacterial circadian clock regulated by oxidized quinone. When KaiA senses oxidized quinone, the model Eqs. (1) and (2) are used. Similarly, for CikA sensing oxidized quinone, the model Eqs. (1) and (3) are used.

3. Results

3.1. Phase shifts brought about by oxidized quinone pulse

As we know, entrainment is a process which aligns the phase of the circadian oscillator with the light–dark cycle. When KaiA or CikA senses oxidized quinone, the phase of the circadian clock is adjusted to achieve entrainment. Generally speaking, the signals alter the phosphatase activities of KaiC and affect the phase of phosphorylation. In the following, we discuss the phase shifts brought about by oxidized quinone pulse sensed by KaiA and CikA at different times. In the following figures, CT0 is their troughs.

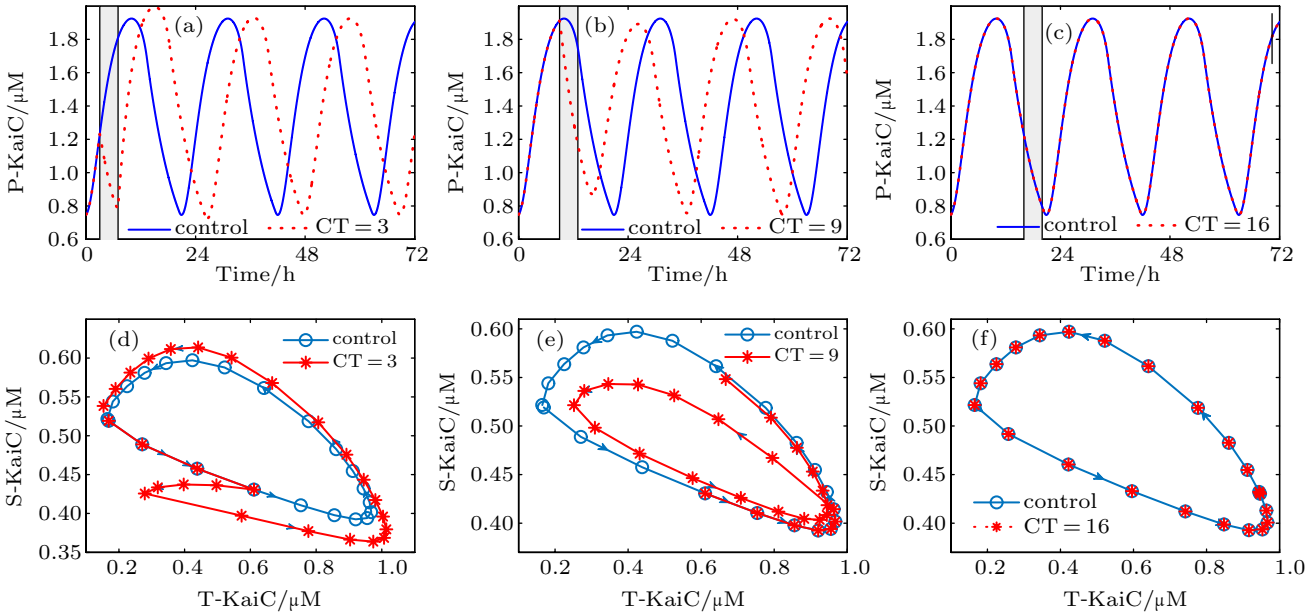

Fig. 1. Phase shifts induced by oxidized quinone pulse sensed by KaiA. A 4 h pulse is added at CT = 3 h [(a), (d)], CT = 9 h [(b), (e)], and CT = 16 h [(c), (f)], respectively. The kinetic trajectories of the total concentration of phosphorylated KaiC (P-KaiC), which is the sum of S-KaiC, T-KaiC and D-KaiC, are shown in (a)–(c). The corresponding orbits are shown and projected onto the T–S plane over a full circadian cycle (d)–(f). The gray bar represents the duration of adding oxidized quinone.

Figure 1 shows the phase shifts when oxidized quinone pulse is added for 4 h at different circadian times (CTs) sensed by KaiA. In the presence of oxidized quinone, KaiA loses its activation by binding oxidized quinone, leading to the dephosphorylation of KaiC.[18] During the phosphorylation process, if the inactivation of KaiA occurs at a low level of phosphorylated KaiC, its phosphorylation level can be recovered after reducing quinone (shown in Fig. 1(a)). On the phase plane diagram, the pulse pulls circadian oscillator to another limit cycle and it returns to the original stable limit cycle after the pulse is removed (shown in Fig. 1(d)). In this situation, the phase appears to be lagging, while it actually undergoes two cycles of phosphorylation and dephosphorylation, which also means a phase advance in a sense. On the other hand, if the inactivation of KaiA occurs at a high level of phosphorylated KaiC, the premature dephosphorylation of KaiC will arise, resulting in an earlier transition to the next part of the cycle (i.e. phase advance, shown in Fig. 1(b)). From the phase plane diagram,

we can see that the pulse makes the orbit reach a smaller limit cycle and it then returns to the original limit cycle after the pulse is removed, which brings about a phase advance (shown in Fig. 1(e)). On the contrary, adding oxidized quinone during the dephosphorylation process of KaiC brings little or no phase shift (shown in Fig. 1(c)) and the orbits remain consistent with and without the pulse (shown in Fig. 1(f)). Overall, KaiA is involved in phase advance and phase delay can not be observed without considering CikA, which is consistent with the biological experiments.[16,18]

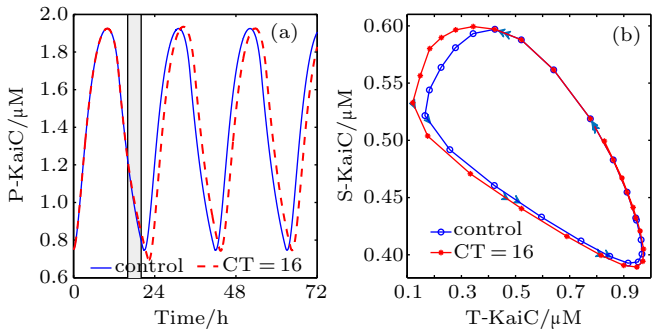

Fig. 2. Phase shift induced by oxidized quinone pulse sensed by CikA. A 4 h pulse is added at CT = 16 h. The kinetic trajectories of P-KaiC before and after the pulse are shown in (a), and the corresponding orbits projected onto the T–S plane over a full circadian cycle are shown in (b). The gray bar represents the duration of adding oxidized quinone.

In Fig. 2, CikA senses oxidized quinone only during the dephosphorylation process, where we can see that it causes phase delay. The presence of oxidized quinone dissociates CikA from KaiB, and KaiA takes over the binding sites instead. Thus, KaiC stays in the dephosphorylation phase for a longer time, resulting in a later transition to the next part of the cycle (shown in Fig. 2(a)). The phase plant diagram in Fig. 2(b) also reveals that the pulse pulls the orbit to a bigger limit cycle and it then returns to the original limit cycle which results in a phase delay.

Making phase response curves (PRCs) is an effective way to study the phase shifts induced by environmental signals added at different circadian times. In order to see the difference between the phase shifts clearly, we draw the PRCs corresponding to KaiA and CikA shown in Fig. 3. Considering Figs. 1–3, one finds that KaiA and CikA are related to phase advance and phase delay, respectively. The phase advance is much larger than the phase delay. In other words, oxidized quinone signals sensed by KaiA produce much larger phase shift than that induced by CikA.

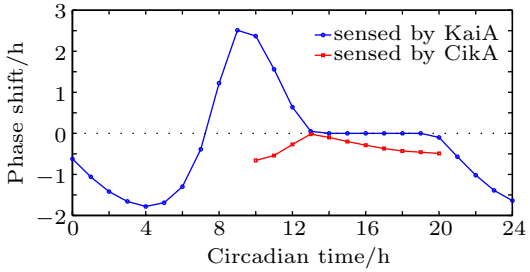

Fig. 3. The phase response curve (PRC) of the circadian clock. The horizontal axis represents the circadian time when oxidized quinone pulse is added. Negative (positive) phase shifts correspond to phase delay (advance). The blue line with circles indicates the situation where KaiA senses oxidized quinone with $Qu = 0.3$ (μM) and the red line with rectangles indicates that CikA senses oxidized quinone with $Qu = 0.6$ (μM). The pulse duration is 1 h.

3.2. Factors influencing phase shifts

Here we discuss the main effects of several factors on PRCs, such as the amount of oxidized quinone, the length of pulse duration (PD) and the inherent period of circadian oscillator.

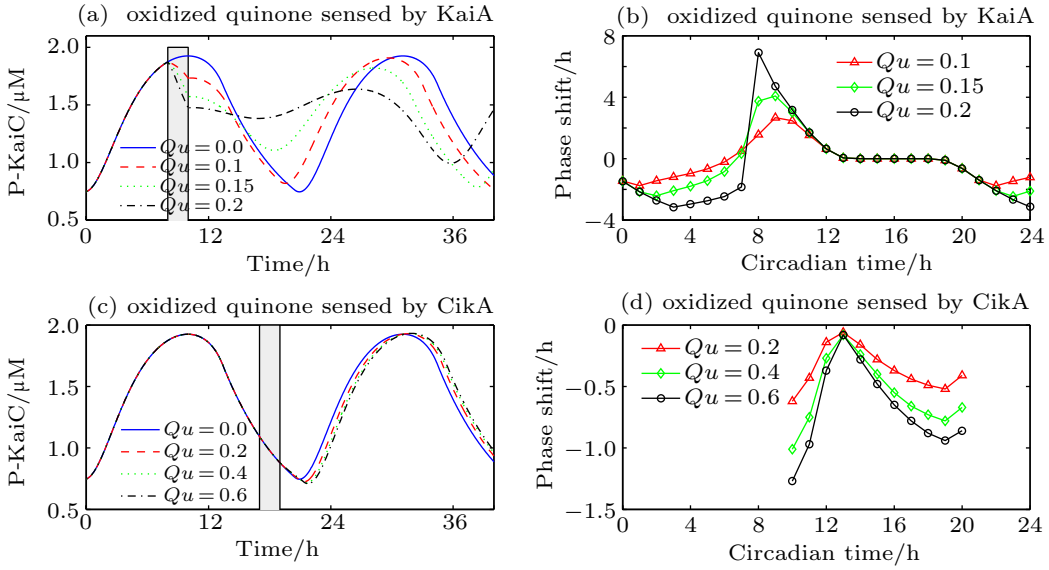

Fig. 4. Effects of varying the amount of oxidized quinone on phase shifts. Oxidized quinone are added at CT = 8 h and CT = 19 h sensed by KaiA [(a), (b)] and CikA [(c), (d)], respectively. The time courses of P-KaiC and PRCs for various amount of oxidized quinone are shown in [(a), (c)] and [(b), (d)], respectively. The gray bar represents the duration of adding oxidized quinone, which is 2 h.

Figure 4 shows the effect of oxidized quinone's amount on phase shifts from two perspectives, P-KaiC time course and PRC. Both the time evolution curves of P-KaiC (shown in Figs. 4(a) and 4(c)) and the PRCs (shown in Figs. 4(b) and 4(d)) tell us that the higher amount of oxidized quinone causes the greater phase shift when the addition time and duration of pulses are constant. From model (1), one knows the high amount of oxidized quinone means more KaiA or CikA senses the signals. Thus the results of Fig. 4 are consistent with the biological conclusion that the amount of the phase advance and phase delay is positively correlated with the concentrations of KaiA and CikA, respectively.[18] Similar results are found in Fig. 5. When the addition time and amount of oxidized quinone are constant, the longer pulse duration brings about the greater phase shift, which also coincides with earlier experiments.[15] In Figs. 4 and 5, CT8 and CT19 are chosen because the addition of oxidized quinone pulses at these two times sensed by KaiA and CikA respectively bring about greater phase shifts, which is more convenient to compare the effects of various factors.

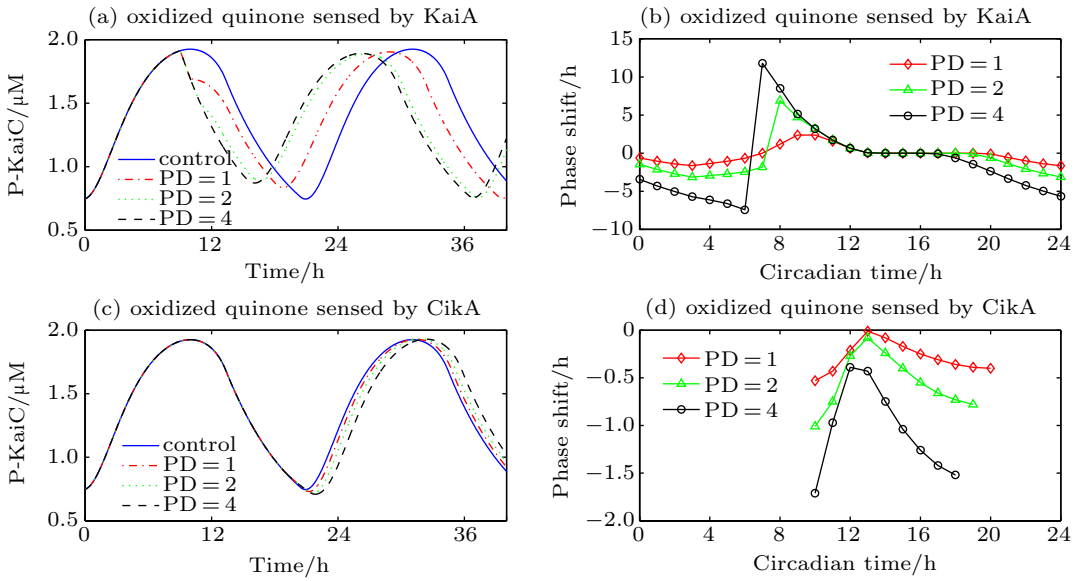

Fig. 5. Effects of varying the pulse duration (PD) of oxidized quinone on phase shifts. Pulses with different durations of oxidized quinone are added at CT = 8 h and CT = 19 h sensed by KaiA [(a), (b)] and CikA [(c), (d)], respectively. The time courses of P-KaiC and PRCs are shown in [(a), (c)] and [(b), (d)], respectively. Here $Qu = 0.2$ (μM) in [(a), (b)] and 0.4 (μM) in [(c), (d)].

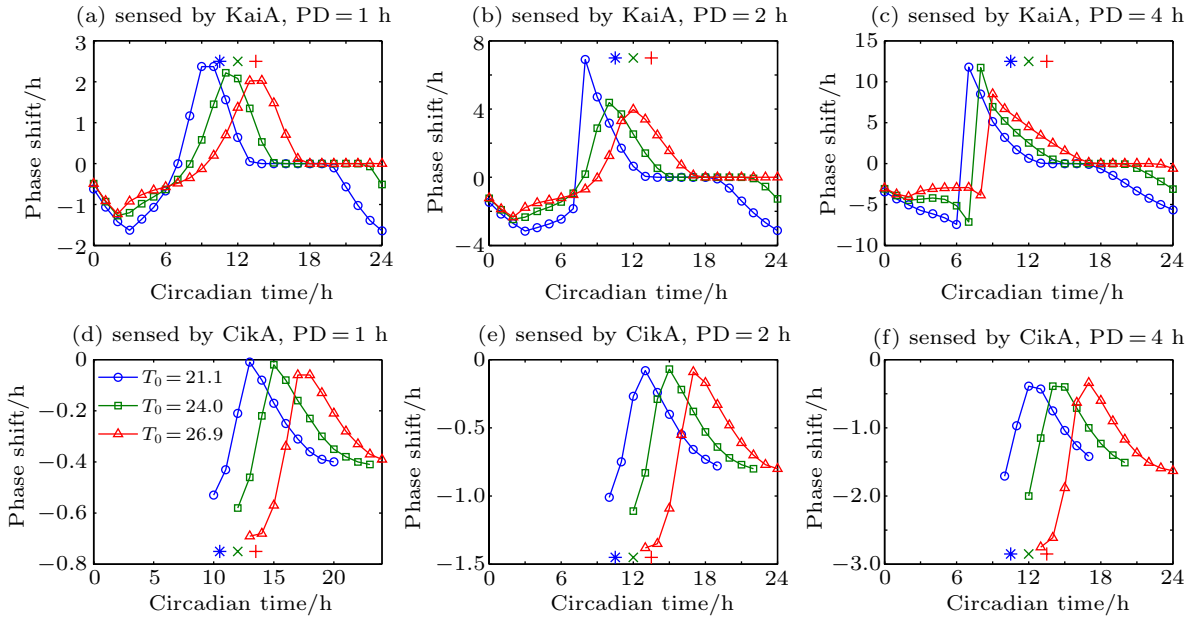

Fig. 6. PRCs of circadian oscillators with different inherent periods (T_0), which are 21.1 h, 24.0 h and 26.9 h, respectively, and [KaiA] = 1.07 (μM), 1.159 (μM) and 1.235 (μM) accordingly. Oxidized quinone is sensed by KaiA (a)–(c) and CikA, which senses oxidized quinone only in the dephosphorylation phase (d)–(f), respectively. The signal duration is 1 h in [(a), (d)], 2 h in [(b), (e)] and 4 h in [(c), (f)]. The asterisk, cross and plus sign represent the peak phosphorylation points of the three circadian oscillators, respectively. Here $Qu = 0.2$ (μM) in (a)–(c) and 0.4 (μM) in (d)–(f).

In order to make clear whether the nature of circadian clock has an effect on entrainment, we study the phase shifts of oscillators with different inherent periods. The results of Fig. 6 show that the effect of inherent periods of circadian oscillators on phase shifts is not obvious. The sensitivity to signal pulses is similar for all of the circadian oscillators with different periods. In Figs. 6(a)–6(c), KaiA senses oxidized quinone signals. The oscillators are most sensitive to signal pulses during the middle of subjective day, when KaiC phosphorylation is increasing and close to its peak, and hardly sensitive during subjective night, when KaiC phosphorylation is decreasing. In Figs. 6(d)–6(f), CikA senses oxidized quinone signals during the dephosphorylation process of KaiC. The oscillators are most sensitive to signal pulses at the beginning of dust and close to the end of dephosphorylation. These findings are consistent with biological observations.[15,18]

3.3. Entrainment of cyanobacterial circadian clock

Quinone is oxidized at the onset of darkness and reduced at the onset of light in cyanobacteria,[16] which reflects the environmental light–dark cycle. Here we explore the difference between the entrainment induced by oxidized quinone cycle sensed by KaiA and CikA.

From Fig. 7, we can see that the cyanobacterial circadian clock can be entrained by oxidized quinone cycle sensed by KaiA or CikA as long as the amount is strong enough. However the time spent by the circadian clock to get entrainment is different. In Fig. 7(a), oxidized quinone is sensed by KaiA which actives the phosphorylation of KaiC directly. When oxidized quinone signal cycle is added, the circadian clock is entrained within one day. In Fig. 7(b), oxidized quinone signal is sensed by CikA which affects the process of KaiC's phosphorylation indirectly. In the presence of oxidized quinone signal cycle, it takes 9 days for the circadian clock to get entrainment, which is much longer than that in the situation of Fig. 7(a). These results are consistent with Fig. 3, which shows KaiA sensing oxidized quinone brings about much larger phase shift than CikA.

In addition, the durations of oxidized quinone in these two cases correspond to two opposite processes, phosphorylation and dephosphorylation of KaiC, respectively. During the presence of oxidized quinone sensed by KaiA, the concentration of P-KaiC decreases and the trough of P-KaiC aligns with the end of oxidized quinone phase, implying the process of KaiC's dephosphorylation (see Fig. 7(a)). Contrarily, during the presence of oxidized quinone sensed by CikA, the concentration of P-KaiC increases and the peak of P-KaiC aligns with the end of oxidized quinone phase, implying the process of KaiC's phosphorylation (see Fig. 7(b)). That is to say, the entrainment phases between the circadian clock and light–dark cycle in Figs. 7(a) and 7(b) are 0 and 12 h, respectively. The difference of entrainment phase corresponds to the different phase shifts brought about by KaiA (phase advance) and CikA (phase delay).

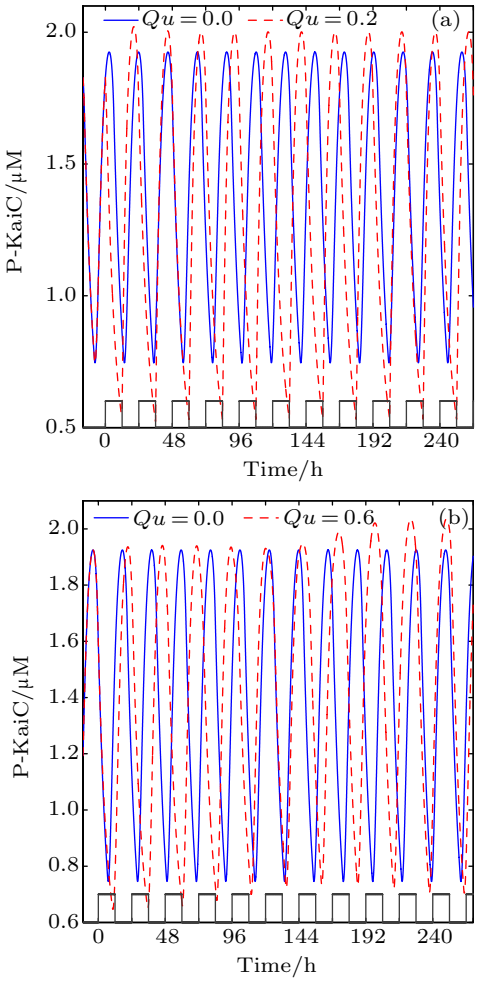

Fig. 7. Entrainment of cyanobacterial circadian clock regulated by oxidized quinone cycle. The blue solid lines and the red dotted lines represent the oscillators without and with oxidized quinone cycle, respectively. The jagged line shows oxidized quinone cycle (12:12), where the high level represents oxidized quinone pulse (12 h) and the low level represents that there is no oxidized quinone (12 h). Oxidized quinone is sensed by KaiA (a) and CikA (b), respectively.

3.4. The mixture effects of KaiA and CikA

In the previous sections, we studied the entrainment of the cyanobacterial circadian clock induced by oxidized quinone sensed by KaiA or CikA. Here, we wonder how KaiA and CikA work together to entrain the circadian clock under oxidized quinone cycle, which is illustrated in Fig. 8.

When the amount of oxidized quinone is not enough, if only one of KaiA or CikA senses oxidized quinone cycle, the phase difference between the circadian oscillator and the oxidized quinone cycle fluctuates over time (shown in Fig. 8(a)), which means that the circadian clock is not entrained. However, if both KaiA and CikA sense the same oxidized quinone cycle as that in (A), the phase difference is stable over time (shown in Fig. 8(b)), which implies that the circadian clock is entrained. We know that KaiA is a component of the core os-

cillator and CikA is an input component of the circadian clock. Thus the results tell us that the combination of core oscillator and input part is conducive to entrainment induced by external environment.

In Fig. 8(b), although the circadian clock is entrained, the entrainment phase is not zero. With the increase of the amount of oxidized quinone, the entrainment phase between the circadian clock and oxidized quinone cycle reduces gradually to zero, as shown in Fig. 8(c). The circadian clock running in various amount of oxidized quinone shows altered oscillator orbits (or limit cycles) (shown in Fig. 8(d)), and a slightly increased free-running period (from 21.1 h to 24 h). Lastly, the clock is entrained to a smoothly varying rhythm with a 24 h period.

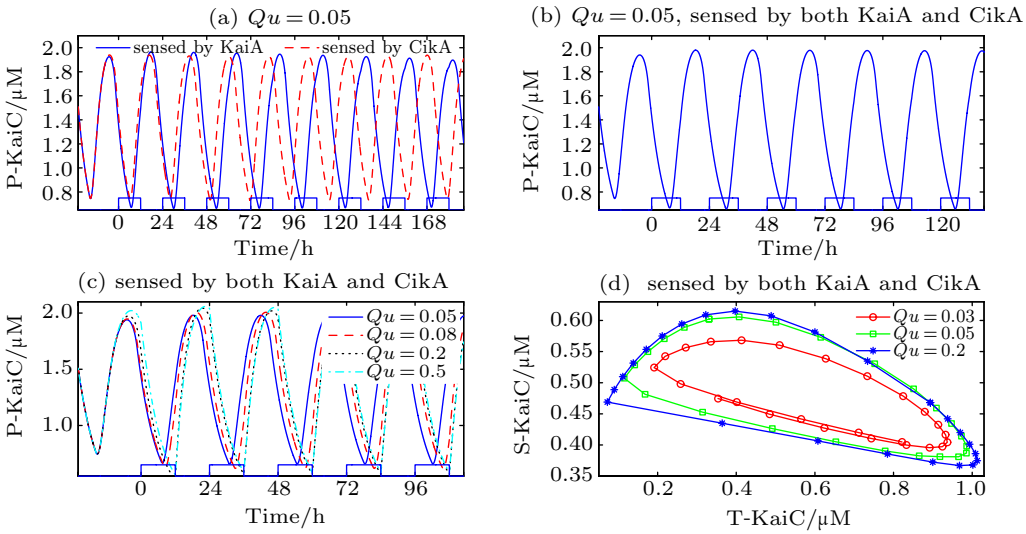

Fig. 8. Mixture effects of KaiA and CikA on the entrainment of circadian clock. The circadian clock can not be entrained when the signal strength of oxidized quinone, sensed by one of KaiA or CikA, is not strong enough (a). However, it can be entrained when oxidized quinone is sensed by both KaiA and CikA (b). The time evolution curves of P-KaiC with varying *Qu* are shown in (c), which shows the entrainment phase difference between the circadian clock and oxidized quinone cycle. (d) Circadian cycles are projected onto T–S plane. The jagged line is the same as that in Fig.7.

4. Conclusion and discussion

Circadian clocks are self-sustained biological oscillators that can be entrained by environmental cues. When cyanobacteria receive an environmental light or dark signal, it is turned into biochemical signals. How these biological signals entrain the cyanobacteria circadian clock is worth exploring. In the present study, we develop a mathematical model based on the results of biological experiments and mainly explored the entrainment mechanisms of the cyanobacterial circadian clock induced by quinone.

In this model, the biological signals are coupled to the clock by both the core oscillator and the input pathway. In detail, the core oscillator protein KaiA and the input component CikA are sensitive to oxidized quinone. We get some meaningful results which concur with biological experiments.

(1) In the presence of oxidized quinone pulse, KaiA and CikA are related to phase advance and phase delay, respectively. KaiA is most sensitive to quinone in the middle subjective day during the increasing phosphorylation process of KaiC, while CikA is most sensitive at dust or just before the end of dephosphorylation of KaiC. CikA is a necessary component for quinone signal input to the clock, because quinone can not produce phase delay without CikA. KaiA and CikA complement the entrainment.

(2) The magnitude of phase shifts is positively correlated with the pulse duration and the amount of quinone. When the amount of quinone is enough, the circadian clock can be entrained by oxidized quinone cycle. A further increase in amount reduces the entrainment phase between the circadian oscillator and the external signals.

(3) The inherent period of circadian clock has not obvious effect on phase shifts. The distance between the time adding pulse and the peak or trough of P-KaiC determines the magnitude of phase shifts.

These findings show the entrainment mechanisms from mathematical view, which provide a theoretical reference for biological work. The mathematical model developed here is meaningful, which can be used to study other biological problems associated with CikA or quinone. Considering CikA as an input component enriches CikA's role in cyanobacterial circadian clock, as most reports focus only on its output action. The results and analytical framework proposed here may provide insight to better understand the entrainment and the dynamic mechanism of the cyanobacterial circadian clock.

In the future, based on the mathematical model developed here, we will further explore the effects of some key factors on the cyanobacterial circadian clock, e.g. time delay or noise. The results show that CikA sensing oxidized quinone is related to phase delay. Next, we are going to consider whether we can

use delay differential equations to describe the regulation of CikA. Noise, coming from external and internal modifications such as temperature and cell division cycles, is an inevitable factor in circadian rhythm. Researchers found that the circadian oscillator in cyanobacteria is resilient to noise, which is a built-in property ensured by the intracellular biochemical network.[28–30] In the near future, we will further explore the dynamical mechanisms of the resilience of cyanobacterial circadian clock to noise.

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