

THE MATHEMATICAL MODEL OF SELF-OSCILLATIONS OF KAI PROTEINS IN INCUBATION SOLUTION

Abstract. A mathematical model of the temperature compensated in vitro oscillation of cyanobacterial KaiC phosphorylation, in a thermodynamically closed system showing circadian rhythmicity, only involving the three Kai proteins (KaiA, KaiB, and KaiC) and ATP is described. It is illustrated, that KaiA- and KaiB-assisted autocatalytic phosphorylation and dephosphorylation of KaiC are the source for self-oscillations of Kai-proteins. This model, based upon autocatalysis instead of transcription-translation negative feedback, shows limit-cycle oscillations with KaiC phosphorylation profiles and has period lengths that are consistent with experimental observations. A frequency control analysis of model demonstrates that the self-oscillations of Kai-proteins are temperature-compensated.

Key words: Mathematical model, biochemical oscillation, **KAI proteins**

Introduction

Circadian rhythms, endogenous oscillations of physiological activities with a period of ~24 h, are found in a wide spectrum of organisms and enhance their fitness in a day/night alternating environment [1]. Among these organisms, cyanobacteria are the simplest organisms that exhibit circadian rhythms [2,3]. Three clock genes *kaiA*, *kaiB* and *kaiC* have been identified in the cyanobacterium, *Synechococcus elongatus* PCC 7942 as essential timekeeping components. A transcription/translation-based autoregulatory loop of *kaiBC* gene expression has been proposed to drive circadian rhythms [4]. In this model, KaiA and KaiC are proposed as positive and negative regulators of *kaiBC* expression, respectively [4].

Recent evidence, however, suggests that the *Synechococcus elongatus* PCC 7942 clock does not absolutely require such a negative feedback oscillator to generate phosphorylation rhythms in a key regulator, KaiC. Temperature-compensated oscillation in KaiC phosphorylation can occur in vivo without *kaiBC* mRNA accumulation, or in the presence of transcription and translation inhibitors [5]. Astoundingly, this self-sustainable and temperature-compensated oscillator can be reconstituted in vitro by incubating purified KaiC, KaiA, KaiB, and ATP [6]. KaiC is an enzyme with autokinase and autophosphatase activities [7,8]. KaiA enhances KaiC function [9] while KaiB diminishes the effect of KaiA on KaiC [8,10].

Results and discussion

We decided to explore this suggestion explicitly by establishing a basic model based on known biological and biochemical observations that did not involve transcription or translation. It is well established that the three Kai proteins interact in all possible combinations [11] and in incubation solution a hexamers of KaiC, dimers of KaiA and tetramers of KaiB occur [10]. Phosphorylation of KaiC hexamers occurs by ATP, and the process is accelerated by a KaiA dimer, and suppressed by KaiB tetramer. In this model we propose that (i)- KaiC hexamers can

exist in three states: unphosphorylated, low (partially) phosphorylated and associated with KaiA dimers, and high (full) phosphorylated and associated with KaiA dimers states, (ii)- the associated with KaiA dimers high phosphorylated KaiC hexamers enhance the phosphorylation of low phosphorylated KaiC, (iii)- unphosphorylated KaiC hexamers enhance dissociation of cluster of Kai A,B,C proteins and (iiii)-KaiB attenuates KaiA-stimulated KaiC phosphorylation.

In Figure 1, we summarize key steps that we reasoned underlie the KaiC oscillator when ATP is provided in excess. The model (Figure 1), represented as a cyclic reaction diagram, contains six processes (R1–R6) depicting the proposed protein-protein interactions and phosphorylation–dephosphorylation events between the Kai proteins. KaiXY denotes the interaction between KaiX and KaiY proteins.

In this model KaiA2HC6p, indicates the cluster of fully phosphorylated KaiC hexamer and KaiA dimer (KaiA2). KaiC6p denotes partially phosphorylated KaiC hexamer and KaiC6-unphosphorylated KaiC hexamers. In process R1, KaiC6 binds KaiA2 (KaiA dimer), forming KaiA2C6p. Since KaiA dimers facilitate the autokinase activity of KaiC6 [9], KaiA2C6 rapidly is converted to the low phosphorylated form KaiA2C6p. In the process R2, KaiA2C6p is converted to high phosphorylated cluster KaiA2HC6p and as in [12], we propose that KaiA2HC6p adopts such a conformation so that it can facilitate the phosphorylation of low phosphorylated KaiA2C6p into KaiA2HC6p by process R3. In the next step, R4, KaiB tetramer associates with KaiA2HC6p to form the cluster KaiA2B4HC6p. This is consistent with experimental data that demonstrate that KaiB interacts with KaiC only after KaiC has bound to KaiA [8,11]. The process R5 expresses the dissociation of cluster KaiA2B4HC6p by interaction with hexamer KaiC6. In the results of this process occurs KaiC dephosphorylation by exchange of subunits among KaiC6 and KaiA2B4HC6p and KaiA and KaiB dissociates from KaiC hexamers. In the process R6 it is simulated the diminishing influence of KaiB tetramers to phosphorylation process of KaiC hexamers by a manner, that KaiB tetramers interact with the low phosphorylated KaiA2C6p complex and therefore, the inactive complex KaiA2B4C6p is formed. This reaction is reversible.

Reaction scheme:



The corresponding to reaction processes (R1–R6), system of differential equations (see formulas 1) consist from the rate equations of the stages R1–R6 in the proposed model:

$$\begin{cases}
 \frac{dKaiC6}{dt} = v_5 - v_1 \\
 \frac{dKaiA2}{dt} = v_5 - v_1 \\
 \frac{dKaiA2}{dt} = v_5 - v_1 \\
 \frac{dKaiB4}{dt} = v_5 - v_4 - v_6 \\
 \frac{dKaiA2C6p}{dt} = v_1 - v_6 - v_3 \\
 \frac{dKaiA2HC6p}{dt} = v_2 + v_3 - v_4 \\
 \frac{dKaiA2B4HC6p}{dt} = v_4 - v_5 \\
 \frac{dKaiA2B4C6p}{dt} = v_6
 \end{cases} \quad (1)$$

Here

$$v_1 = k_1 * KaiA2 * KaiC6$$

$$v_2 = k_2 * KaiA2C6p$$

$$v_3 = k_3 * KaiA2HC6p * KaiA2C6p$$

$$v_4 = k_4 * KaiB4 * KaiA2HC6p$$

$$v_5 = k_5 * KaiA2B4HC6p * KaiC6$$

$$v_6 = k_{+6} * KaiB4 * KaiA2C6p - k_{-6} * KaiA2B4C6p$$

and k_i ($i=1-6$) rate constants.

In the model there are 3 conserved entities:

$$[KaiA2C6p] + [KaiB4] + [KaiA2] + [KaiA2HC6p] = const_1$$

$$-[KaiA2] + [KaiC6] = const_2$$

$$[KaiB4] + [KaiA2B4HC6p] + [KaiA2B4C6p] = const_3$$

Consequently this means, that size of corresponding stoichiometric matrix of reaction scheme is 7x6, and rank of matrix is 4.

It is well known that autocatalytic stages in dynamic systems destabilize a steady state. From this point of view, processes R3 and R5 draw attention, since they are autocatalytic and can serve as sources of sustained oscillations. The bifurcation analysis of the model carried out by us shows that, the source of the oscillations is the autocatalytic reaction R3 of the high phosphorylated KaiC6–KaiA2 complex (KaiA2C6p, Figure 1) coupled with subsequent removal of the autocatalytic species and regeneration of dephosphorylated KaiC6 (reaction R5). The suggested autocatalytic formation of KaiA2C6p is typical for the kinetics of protein kinases showing autophosphorylation [15]. The autocatalysis permits sustained oscillations around a nonequilibrium unstable steady state as long as enough ATP is present to drive the autocatalytic phosphorylation, which keeps the system far from steady state. However, in the presence of the autocatalytic step R5 but in the absence of the autocatalytic step R3, no sustained oscillations appear possible, but damped oscillations may be observed in the approach to a nonoscillatory steady state. The

stage R5 describes autocatalytic dephosphorylation of the complex KaiC6 by the "exchange" interaction of nonphosphorylated KaiC6, suggested in [13].

The in vitro KaiC oscillator is based on the KaiA/KaiB assisted phosphorylation/dephosphorylation of KaiC at constant protein levels. Therefore, unlike circadian oscillators based on transcription and translation [16], degradation reactions do not play any significant role in the in vitro KaiC oscillator.

The model's differential equations (1) were solved numerically using the software programs SBW (authors H.Sauro et al., [http:// www.sbml.org](http://www.sbml.org)) and results of numerical solution of (1) with following parameter values: $k_1 = 0.01\mu M^{-1}h^{-1}$, $k_2 = 0.001\mu M^{-1}$, $k_3 = 8.60\mu M^{-2}h^{-1}$, $k_4 = 6.25\mu M^{-1}h^{-1}$, $k_5 = 0.2\mu M^{-1}h^{-1}$, $k_{-6} = 0.0005\mu M^{-1}h^{-1}$, $k_{+6} = 0.01\mu M^{-2}h^{-1}$, $[KaiA2]=0.703\mu M$, $[KaiB4]=0.337\mu M$, $[KaiC6]=1.436\mu M$, $[KaiA2C6p]=0.255\mu M$, $[KaiA2B4HC6p]=0.027\mu M$, $[KaiA2HC6p]=0.005\mu M$, $[KaiA2B4C6p]=0.152\mu M$ are illustrated in figure 2 (a, b, c).

It should be noted that expansion of model by addition of reactions of formation of complexes *KaiA2*, *KaiB4*, and *KaiC6* from respectively monomers *KaiA*, *KaiB*, and *KaiC* doesn't change a dynamic picture of oscillations, for this reason we offer more simplified model.

Frequency and amplitude control analysis and temperature compensation.

Temperature compensation of frequency of oscillations is an essential property of clockwise biochemical oscillations, which means that in some living oscillatory biochemical processes the frequency (or period) remains as constant (more exactly, approximately constant) in physiologically allowed range of the temperature change. For the verification of the temperature compensation of above described model, it is necessary metabolic control analysis for frequency in model. From the point of view of metabolic control analysis, temperature compensation means, that a response coefficient of frequency relatively to temperature change must be equal or near to zero. Consequently, this fact may be a very good criteria for the verification of oscillatory model for the temperature compensability.

It is well known, that each rate constant depends on temperature and this dependence obeys the Arrhenius equation,

$$k_i = A_i e^{-\frac{E_i}{RT}}, \quad (2)$$

where E_i is the activation energy, R is the gas constant, and T is the temperature in Kelvin. A_i is the pre-exponential factor, which is also treated as a constant. Therefore a change of temperature of reaction system exponentially influences on rate constant, a namely, the increase of temperature holds an exponentially increase of all rate constants.

For the quantitatively determine a response of frequency on change of temperature, we present cyclic frequency as complex function from temperature as: $\omega(T) = \omega(k_i(T))$ and

$$\frac{\partial \omega}{\partial T} = \sum_{i=1}^n \frac{\partial \omega}{\partial k_i} \frac{\partial k_i}{\partial T}$$

165 From here

166

$$167 \frac{T}{\omega} \frac{\partial \omega}{\partial T} = \sum_{i=1}^n \frac{k_i}{\omega} \frac{\partial \omega}{\partial k_i} \frac{T}{k_i} \frac{\partial k_i}{\partial T} \quad (3)$$

168

169 Here,

170 $C_{k_i}^{\omega} = \frac{k_i}{\omega} \frac{\partial \omega}{\partial k_i}$ is control coefficient for frequency on rate constant. Then

171 $C_T^{\omega} = \frac{T}{\omega} \frac{\partial \omega}{\partial T}$ will be a responcity coefficient of frequency on the temperature and

172 $C_T^{k_i} = \frac{T}{k_i} \frac{\partial k_i}{\partial T}$ will be a responcity coefficient of i -th rate constant on the temperature.

173 From Arrhenius equation (2) we receive that

$$174 C_T^{k_i} = \frac{E_i}{RT} \quad (4)$$

175 By regarding (4) in (3)

176

$$177 C_T^{\omega} = \frac{1}{RT} \sum_{i=1}^n C_{k_i}^{\omega} E_i \quad (5)$$

178

179 Formula (5) shows, that temperature compensation requires that one or several of the
180 frequency control coefficients need to be negative, because R, T and activation
181 energies are positive. Consequently, the in the temperature compensated oscillators
182 should be a some reactions that have opposing effects on the frequency relatively to
183 temperature changes. Temperature compensation can occur within a certain
184 temperature interval, whenever the activation energy (E_i) weighted sum of the control
185 coefficients is near to zero.

186 The frequency control coefficients in (5) we calculate by a manner as described in
187 [17,18] and obtained values are listed in tabl.1.

188

189 **Table 1. Control coefficients of the reaction steps on the model.**

190

	$C_{k_i}^{\omega}$	$C_{k_i}^{A_{KaiA2}}$	$C_{k_i}^{A_{KaiB4}}$	$C_{k_i}^{A_{KaiC6}}$	$C_{k_i}^{A_{A2B4HCp}}$	$C_{k_i}^{A_{A2HC6p}}$	$C_{k_i}^{A_{A2B4Cp}}$	$C_{k_i}^{A_{A2C6p}}$
k_1	0.376	6.32	6.73	6.53	6.74	6.64	6.71	6.56
k_2	0.017	-6.07	-6.15	-6,11	-6.02	-6.07	-5.71	-4.73
k_3	0.778	-5.40	-5,14	-5,25	-5.69	-5.45	-5.85	-5.27
k_4	-0.361	7.51	6,82	7.25	6.64	6.78	6.29	6.36
k_5	0.187	-2.84	-2,54	-2.84	-3.12	-2.01	-2.58	-3.10
k_{+6}	-0.029	0,481	0,46	0.76	1.36	0.53	1.14	0.53
k_{-6}	0.029	-0,002	-0,18	-0,34	0.09	-0.42	0.00	-0.35

Sum	0.997	-0.001	0.00	0.00	0.00	0.00	0.00	0.00
Sum*	1.0001	0,0000	0,0000	0,0001	0,0000	0,0000	0,000	0,000
*All kinetic parameters are increased simultaneously by 5% and the total change of frequency ω and amplitudes A_i is determined								

191

192 As it is visible from Tab.1, model has temperature -compensate mechanism and may
 193 be temperature-compensated. Really, in model the process R4 (rate constant k_4), (In
 194 process R4, *KaiB4* associates with *KaiA2HC6p* to form the ternary complex
 195 *KaiA2B4HC6p*) has a negative frequency control coefficient.

196 Such result reasonably from the point of view of molecular kinetics as the increase
 197 in temperature has to complicate formations of a complex.

198 From the tab.1 it is visible, that in model there is one more stage with negative
 199 frequency control coefficient. This is reaction of inhibition of complex *KaiA2C6p*
 200 phosphorylation by protein *KaiB*, (process R6) that also corresponds to the fact that
 201 *KaiB* diminishes the effect of *KaiA* on *KaiC* [8,10], though has considerably small
 202 value in comparison with frequency control coefficient of stage R4.

203 From the theory of oscillations it is known that values of an amplitude of self-
 204 oscillations are defined by internal parameters (the rate constants in our case) self-
 205 oscillatory system. Since the change of temperature influences to rate constants on
 206 Arrhenius formula, it is arise an interesting question about the influence of
 207 temperature change on the amplitude of oscillations of Kai proteins. In order to
 208 answer to this question we carried out the control analysis of amplitudes of
 209 oscillations on the way described in [18] and the received results are listed in Tab.1.

210 From the presented data in the Tab.1 it is visible that for amplitudes it is also carried
 211 out the known theorem of summation of concentration control coefficients and as in
 212 case of frequency, here too there are negative control coefficients. By analogy with
 213 frequency, this fact allows to think of "a temperature compensation" of amplitude,
 214 but in difference from frequency, we don't know experimental data of influence of
 215 change of temperature on amplitude of biochemical self-oscillations.

216 As it is in high mentioned expansion of model by addition of reactions of formations
 217 of complexes *KaiA2*, *KaiB4*, and *KaiC6* from respectively monomers *KaiA*, *KaiB*, and
 218 *KaiC* doesn't change a dynamic picture of oscillations. However, the advantage of
 219 "minimal" models like the one presented here is that they are easily applied and
 220 extendable. Our model suggests that the in vitro *KaiC* phosphorylation rhythm is a
 221 circadian oscillator that is driven by autocatalysis. In it, we see a new relationship
 222 between nonlinear chemical dynamics and chronobiology.

223 **Conclusion**

224 The temperature compensated in vitro oscillation of cyanobacterial *KaiC*
 225 phosphorylation, the first example of a thermodynamically closed system showing
 226 circadian rhythmicity, only involves the three Kai proteins (*KaiA*, *KaiB*, and *KaiC*)
 227 and ATP. In this paper, we describe a model in which the *KaiA*- and *KaiB*-assisted
 228 autocatalytic phosphorylation and dephosphorylation of *KaiC* are the source for

circadian rhythmicity. This model, based upon autocatalysis instead of transcription-translation negative feedback, shows temperature-compensated circadian limit-cycle oscillations with KaiC phosphorylation profiles and has period lengths and rate constant values that are consistent with experimental observations.

This phenomenon called "temperature compensation" - the most important distinctive property of biochemical "clock" which, in our opinion, has to take place in biochemical systems for the reason that oscillations in biological processes are also carriers of the biophysical information coded by characteristics of oscillation. Protection of this information against distortion by casual temperature fluctuations is necessary for live systems. Here we conclude that at temperature compensated biochemical reaction there have to be stages which are opposite reacting on influence of temperature on self-oscillation frequency.

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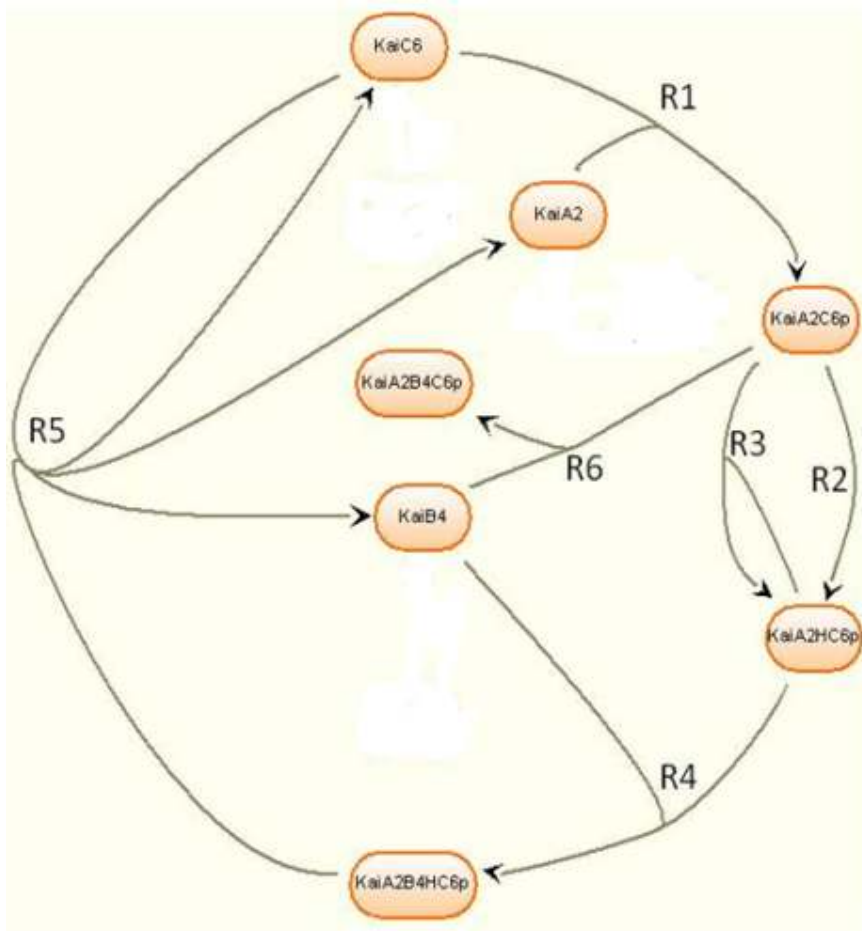
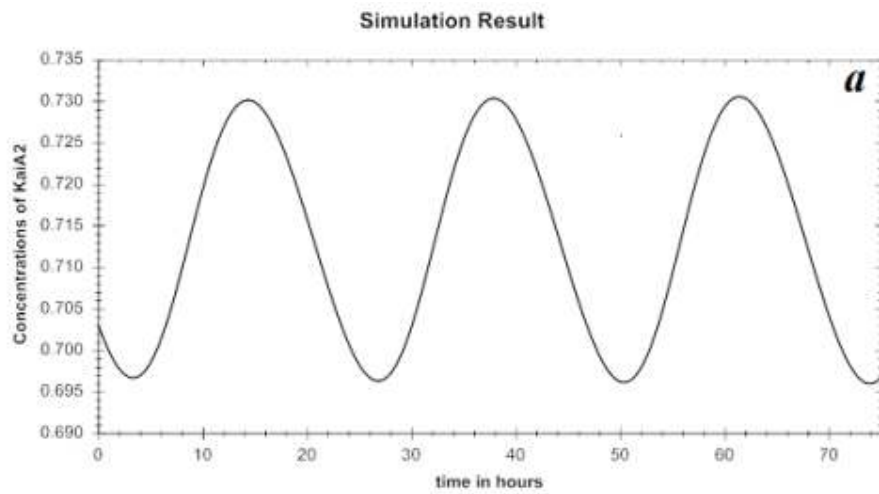
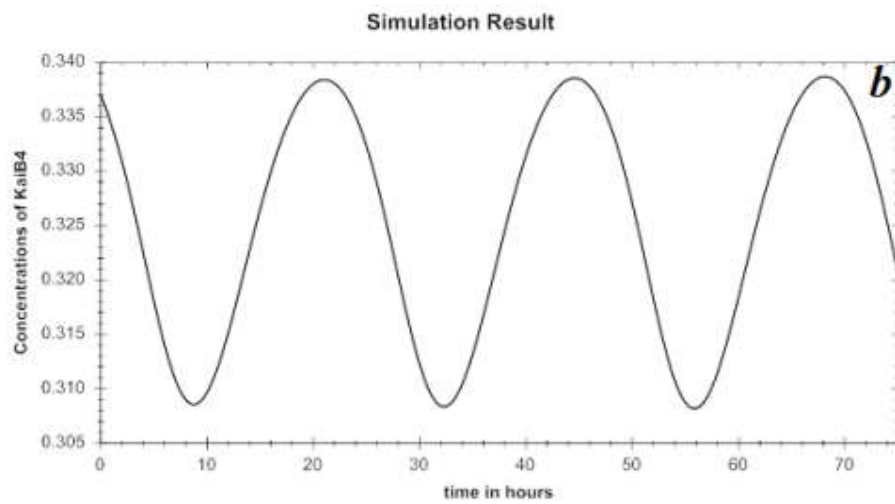


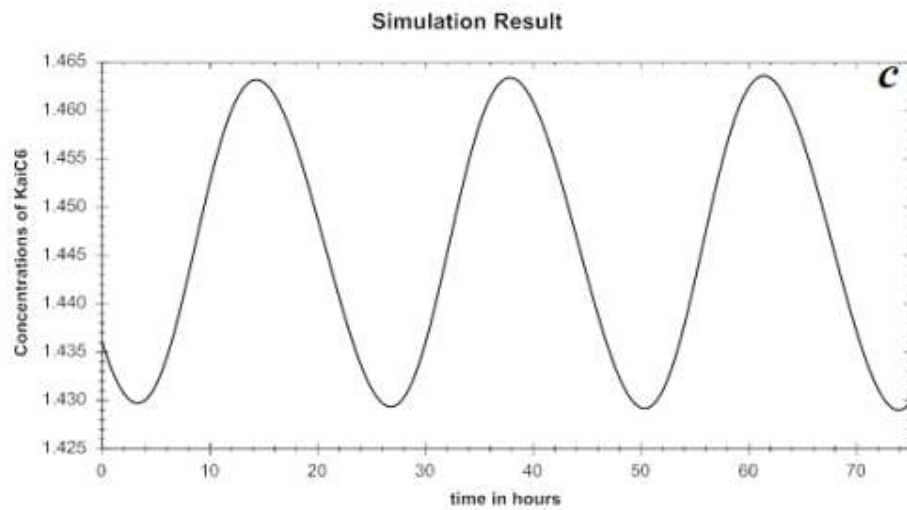
Figure 1. A dynamical model of in vitro rhythmic KaiC6 phosphorylation in incubation solution (See text for description).



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298 Figure 2. Self-oscillations of concentrations of Kai proteins in incubation solution:
 299 (a) KaiA2,
 300 (b) KaiB4 and
 301 (c) KaiC6 proteins