Modeling temperature entrainment of circadian clocks using the Arrhenius equation and a reconstructed model from *Chlamydomonas reinhardtii*

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Abstract Endogenous circadian rhythms allow living organisms to anticipate daily variations in their natural environment. Temperature regulation and entrainment mechanisms of circadian clocks are still poorly understood. To better understand the molecular basis of these processes, we built a mathematical model based on experimental data examining temperature regulation of the circadian RNA-binding protein CHLAMY1 from the unicellular green alga *Chlamydomonas reinhardtii*, simulating the effect of temperature on the rates by applying the Arrhenius equation. Using numerical simulations, we demonstrate that our model is temperature-compensated and can be entrained to temperature cycles of various length and amplitude. The range of periods that allow entrainment of the model depends on the shape of the temperature cycles and is larger for sinusoidal compared to rectangular temperature curves. We show that the response to temperature of protein (de)phosphorylation rates play a key role in facilitating temperature entrainment of the oscillator in *Chlamydomonas reinhardtii*. We systematically investigated the response of our model to single temperature pulses to explain experimentally observed phase response curves.

Keywords Circadian oscillator · Temperature compensation · Temperature entrainment · Phase response curves · *Chlamydomonas reinhardtii*

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1 Introduction

Most organisms employ circadian clocks to anticipate daily variations in their natural environments. Circadian clocks are self-sustained oscillators that can be entrained by external time cues like light–dark or temperature cycles to match local time [1]. The rhythm persists in the absence of such stimuli with a period of approximately 24 h. Thus, circadian clocks are generally thought to resemble limit-cycle oscillators.

Whereas light entrainment is usually integrated via specific components, the photoreceptors, temperature permanently influences all processes within an organism. Thus, it is difficult to study temperature regulation of circadian clocks separately by *in vivo* experiments and the underlying mechanisms are poorly understood. Although the clock can be entrained by temperature cycles, its free-running period is relatively constant within a broad range of physiological temperatures. This phenomenon is called temperature compensation [2, 3]. To our knowledge, the relationship between these two effects has not yet been thoroughly investigated.

Based on recently published experimental data, we reconstructed a model of the circadian clock of the green alga *Chlamydomonas reinhardtii*. In this organism, an RNA-binding protein CHLAMY1 is known to bind UG-repeat sequences in the 3' untranslated region (UTR) of target mRNAs [4]. Introduction of UG-repeats induces circadian expression in reporter constructs [5]. As CHLAMY1 is furthermore involved in acrophase and period maintenance [6], it is a likely component of the circadian oscillator in *C. reinhardtii*. Additional candidates for the circadian oscillator of this organism have been proposed by several researchers [7–9]. However, their interactions have not yet been analyzed and no clock model has been proposed so far.

CHLAMY1 consists of two subunits, C1 and C3. C1 phosphorylation and the amount of C3 as well as the activity of *c3*-promotor-driven luciferase reporter constructs vary in a temperature-dependent manner [10]. Both processes might be co-regulated, as phosphorylation of C1 as well as protein levels of C3 and reporter activity are reduced at higher temperature. Furthermore, overexpression or silencing of C1 results in an even more pronounced overexpression or silencing of C3, respectively [6, 11]. So far it is not known whether temperature compensation or entrainment of the circadian clock or even both processes are influenced by CHLAMY1, but there are indications from *per1* mutant studies that at least temperature entrainment is altered in mutants affecting C1 phosphorylation [10]. Furthermore, a role of phosphorylation in temperature regulation has been shown in experiments on other organisms [12, 13].

Based on a schematic model proposed by Seitz et al. [11], we build a mathematical model of the circadian clock of *C. reinhardtii*. We are able to show that the proposed additional feedback of C1 on the *c3*-E-Box region is not necessary to achieve the co-regulation of C1 and C3 expression found experimentally [14]. In our model, we describe the temperature dependence of the reaction rates by the Arrhenius equation. Ruoff and coworkers proposed that temperature compensation is an intrinsic property of circadian clocks [15] and showed that it is always possible if suitable activation energies are chosen. This is known as the antagonistic principle. Within certain temperature ranges, the Arrhenius equation can be used for enzymatic reactions, while it fails at higher temperatures, when enzymes start denaturing.

We achieve temperature compensation in our model using the method proposed by Ruoff et al. [15] where we additionally constrained the activation energies to the range usually

observed for biochemical processes. Furthermore, we investigate properties of temperature entrainment and show that the increased phosphorylation of C1 at lower temperature is required to achieve proper temperature entrainment of our model. Additionally, we compare temperature entrainment by sinusoidal and rectangular temperature cycles and show that the range of periods that allow proper entrainment of the model is much more restricted for rectangular curves. Finally, we simulate the response of our model to single temperature pulses and obtain a phase response curve (PRC) that is in good agreement with our predictions and with experimental observations. We furthermore disassemble the overall PRC to identify the processes that determine the shape of this curve.

2 Results

Although *Chlamydomonas reinhardtii* has been a model organism for the investigation of circadian rhythms for quite some time, no model of its circadian clock exists up to now. Here, we reconstruct a mathematical model for the circadian oscillator based on experimental data (see Fig. 1). The model, discussed in more detail below, is based on the Goodwin oscillator [16], which involves a negative feedback loop. In addition, it contains a complex formation and a phosphorylation cycle.

Fig. 1 Schematic model of the circadian oscillator of *C. reinhardtii*. The indices used in the ODEs coincide with the corresponding arrow labels

In *C. reinhardtii*, the circadian RNA-binding protein CHLAMY1 has been shown to induce circadian expression in reporter constructs by binding to (UG) $_{\geq 7}$ -repeats in their 3'-UTR [5]. The phosphorylation of CHLAMY1 subunit C1 is reduced at higher temperature [10]. Changing the expression levels of either subunit of CHLAMY1 causes arrhythmicity and phase shifts, respectively [6]. As *c3*-mRNA as well as the C3 protein cycle over the course of the day, although with very low amplitude at the protein level [10, 17, 18], it is likely that C3 is part of a negative feedback loop that causes the cycling of its components. In contrast, *c1*-mRNA and protein levels are relatively constant. Therefore, we placed C1 outside the negative feedback loop to reflect experimental data (see Fig. 1). The implemented phosphorylation of C3 has been postulated but not been verified experimentally yet [11, 14]. The resulting model resembles the schematic model proposed by Seitz et al. [11]. However, in that article an additional positive feedback from C1 to C3 was proposed. As shown in Fig. 2, this additional feedback is not necessary to simulate the correlation of C1 and C3 expression observed experimentally. This is possible because C1 binds to C3 and thus sequesters C3 for complex formation. This results in an indirect positive feedback on C3-expression.

The stoichiometry of C1 and C3 in the complex is unknown so far. However, at the beginning of the night CHLAMY1 most efficiently binds to (UG) $_7$ -repeats [19]. At the same time, a rather low-molecular-weight complex comprising C1 and C3 exists [17]. Thus, the functional CHLAMY1-complex does not seem to contain more than three components. As experimental data show that the upregulation of C1 results in an even stronger upregulation of C3, a high C3 to C1 ratio is required. Altogether, the scenario indicates a complex composition of two molecules of C3 and one molecule of C1.

The scheme of the mathematical model used throughout this article is depicted in Fig. 1. The mathematical model and the corresponding parameter settings are described in the

Fig. 2 We varied the translation rate (*continuous line*) or the phosphorylation rate (*dotted line*) of C1 and calculated the amount of *c3*-mRNA at its maximum. Both rates correlate with the amount of *c3*-mRNA in our model

Methods section. Our model is able to reproduce the correlation of C1 and C3 expression as well as the correlation of C1-phosphorylation with C3-expression observed experimentally (Fig. 2).

2.1 Temperature compensation

As Ruoff and coworkers showed [15] on the basis of the Arrhenius equation and concepts from metabolic control analysis [20], it is always possible to achieve temperature compensation in oscillatory models by choosing suitable activation energies. This antagonistic principle implicates that there are processes that increase the period of the oscillator while others simultaneously decrease it. This can be analyzed by determining the period control coefficients of a model as described in Section 4. Figure 3 shows the period control coefficients of our model oscillator. The transcription rate v_1 and the mRNA degradation v_2 have the highest and lowest control coefficients, respectively. A positive control for transcription has been observed, e.g., for the mammalian circadian oscillator [21], and the importance of mRNA degradation for period control has already been demonstrated in 1999 [22]. Furthermore, the control coefficients are in a similar range as in other circadian clock models [23].

To avoid the assumption of non-physiological activation energies, we employed the knowledge that biochemical processes usually comprise a Q_{10} of 2 to 3, but can sometimes have a Q_{10} up to 5 [24]. The factor Q_{10} is a measure of the fractional change of a reaction rate as a consequence of increasing the temperature by 10 K. We furthermore assumed that reaction rates and maximal velocities v_i are the only temperature-dependent parameters of our model. The Michaelis-Menten constants k_i have been considered to be temperature-independent. This holds as Michaelis-Menten constants are ratios of rate constants and

Fig. 3 Period control coefficients of reaction rates were calculated as described in Section 4

furthermore are specific for a certain reaction. Therefore, usually vary slightly, if at all with temperature.

The relation between reaction rates at a basal temperature, their temperature dependency and the Q_{10} are defined by the well-known Arrhenius equation (see Section 4). Thus, if we assume a Q_{10} of 2 to 3, the activation energies are between 50 and 80 kJ/mol. As standard activation energy we applied 67 kJ/mol, corresponding to an average Q_{10} of 2.5. As basal temperature we used 291 K (18°C) as this corresponds to the lower temperature applied in the experimental investigation of C1 phosphorylation.

We initially restricted ourselves to three distinct activation energy levels. The activation energies of rates having a negative period control coefficient were set to the lower limit of activation energies, corresponding to 50 kJ/mol. Reactions having a positive period control coefficient were modeled with high activation energies. As we solely applied this to the reactions of the negative feedback loop and the complex formation, the upper limit of activation energies had to be adjusted to 84 kJ/mol to achieve proper temperature compensation. The expression levels of C1 do not change with temperature. Thus, we used a standard activation energy of 67 kJ/mol for the translation and degradation of C1. These processes have period-control coefficients with different signs and similar absolute values. Thus, applying high and low activation energies as for the negative feedback loop would lead to temperature dependent changes in the expression of C1. However, if we apply the same activation energy and thus the same Q_{10} for both processes, C1 expression is relatively constant with temperature (not shown) as observed experimentally.

The control coefficients of the phosphorylation and the dephosphorylation reactions as well as for the degradation of phosphorylated C1 are relatively low. Moreover, we wanted to separately investigate the role of the experimentally observed increase in C1 phosphorylation at low temperature. Therefore, we kept the activation energy of those processes initially at 67 kJ/mol.

If we apply the activation energies according to the rules stated above, we obtain a temperature compensation of our oscillator with a period Q_{10} of approximately 1.14. This is

Fig. 4 **a** Temperature-dependent changes of the period of the oscillator between 288 and 310 K for different activation energies for the phosphorylation (E_A^p). The oscillator is temperature-compensated with a period Q_{10} of 1.21 for $E_A^p = 40$ kJ/mol (dashed line) and with a period Q_{10} of 1.14 for $E_A^p = 67$ kJ/mol (dotted line). If all activation energies are equally set to 67 kJ/mol, the oscillator is not temperature-compensated and has a period Q_{10} of 2.51 (continuous line). **b** The concentration of c3-mRNA cycles robustly between approx. 288 and 310 K with a slight decrease at high temperature. Below 288 K, the oscillations are damped, and decrease below 281 K. This is reflected by a reduced c3-mRNA amplitude

clearly within the range of compensation experimentally reported for circadian oscillators. Figure 4a shows the temperature dependency in comparison to the uncompensated model where all activation energies are set to 67 kJ/mol. We did not test temperatures below 288 K due to the fact that the oscillator becomes damped for lower temperatures (Fig. 4b). This resembles experimental observations in *Gonyaulax polyedra* (*Lingulodinium polyedra*) where oscillations disappear at low temperature but can be restored after application of higher temperature [25].

2.2 Temperature entrainment

To our knowledge, experimental data on temperature entrainment are rare (see [26–28] for recent examples) and only a few modeling approaches have dealt with this topic so far. Ruoff and Rensing [29] have simulated the response of their model to two different temperature cycle periods. However, the amplitude of the system variables in their simulation is volatile. Therefore, it is possible that they observed quasiperiodic behavior. As Takeuchi et al. [30] showed in their model, for certain amplitudes of temperature changes, the oscillator displays a nearly periodic behavior with a period close but not equal to the period of the temperature cycle. To avoid this problem, we calculated the length of every

Fig. 5 We varied the activation energy for the phosphorylation (E_A^p) and dephosphorylation (E_A^{dp}) of C1 and **a** tested the entrainment of our model to temperature cycles with an amplitude of 10 K and a period of 24 h (black—proper entrainment, grey—period doubling, and white—not entrainable) **b**. Additionally, we calculated the ratio between the amount of phosphorylated C1 at 291 K and 301 K. Contours were drawn at ratios 2, 1.6, and 1.2. In part **c**, the correlation between the two activation energies and the period Q_{10} of our model oscillator is shown. Contours were drawn at 1.2, 1.15, and 1.1. In **d**, example time courses are given for entrainment (upper panel) and period doubling (lower panel). For these time courses, E_A^{dp} was set to 67 kJ/mol and E_A^p was set to 40 kJ/mol (upper panel) or 60 kJ/mol (lower panel)

cycle to determine whether the oscillator has been entrained to the temperature cycles, whether period doubling occurs or whether the oscillator is not entrainable at all (see Section 4 for detailed description).

Motivated by the question of the role of temperature dependent C1 phosphorylation for temperature entrainment and compensation, we varied the activation energy for both processes between 35 and 75 kJ/mol. As shown in Fig. 5, the entrainment is relatively independent of the activation energy for the dephosphorylation reaction (E_A^{dp}). On the other hand, effective entrainment could exclusively be achieved with a maximal activation energy of 50 kJ/mol for the phosphorylation reaction (E_A^p). The exact value slightly varies with the value of the activation energy for the dephosphorylation reaction.

We furthermore calculated the amount of phosphorylated C1 at 291 K (18°C) and 301 K (28°C) and plotted the ratio between the amount of phosphorylated C1 at both temperatures (see Fig. 5b). Interestingly, the contour line drawn for a ratio of 1.6 correlates with the border of proper entrainment in Fig. 5a. Experimentally, a ratio of about 2 has been determined. Hence, increased phosphorylation of C1 at lower temperature seems to be important for appropriate entrainment in our model.

In Fig. 5c we show how the activation energies of the phosphorylation and dephosphorylation of C1 influence the period Q_{10} values of our oscillator. As the period control coefficient of the phosphorylation reaction is relatively low, the impact of changes in the activation energy of the phosphorylation reaction on temperature compensation is also low and the period Q_{10} is within the physiological range for all values tested. Still, the temperature compensation is slightly stronger with higher activation energies for the phosphorylation and lower activation energies for the dephosphorylation of C1. In contrast, the entrainment requires the opposite behavior. Thus, at least in this case, the mechanisms for temperature entrainment and compensation are contradictory. Nevertheless, temperature compensation is required to some extent for entrainment as the uncompensated oscillator (all $E_A = 67$ kJ/mol) cannot be entrained with temperature cycles of any amplitude or period.

Finally, we tested the entrainment of our oscillator with different amplitudes and periods. For the phosphorylation of C1 we used an activation energy of 40 kJ/mol. For the

Fig. 6 Depending on the temperature amplitude, our model oscillator can be entrained to periods around 24 h. The entrainment was simulated for 1,000 modeling hours either with **a** sinusoidal or **b** rectangular temperature curves. The latter resembles the standard experimental procedure but shows a limited range of entrainment compared to sinusoidal temperature cycles (*black*—proper entrainment, *grey*—period doubling and *white*—not entrainable)

Fig. 7 **a** Phase response curve for temperature shifts of 5 K for 1 h. Single reaction rates have been simulated to be perturbed by temperature pulses at different circadian time points (*thin and dotted lines*) to identify those with the highest contribution to the overall PRC gained by perturbing all reaction velocities simultaneously (*thick solid black line*). **b** Time courses of all species except the complex under free-running conditions. Calculation of circadian time is based on the relative position of the *c3*-mRNA peak as described by Johnson et al. [1]

dephosphorylation we applied the standard activation energy, as this best reproduces the difference observed in phosphorylation levels and allows robust entrainment to different amplitudes and periods as shown in Fig. 6a. For sinusoidal temperature cycles, one observes a tongue-shaped entrainment region that is known as the Arnold tongue [31]. In contrast, the entrainment with rectangular temperature curves, which resembles the standard experimental procedure, shows a limited range of entrainment compared to sinusoidal temperature cycles (Fig. 6b). Similar differences have been shown for simulations of light entrainment in a mathematical model for *Neurospora crassa* [32] and experimentally in hamsters by Boulos et al. [33]. Both investigations showed that the range of entrainment cycles is larger if the dawn and dusk transitions are gradual, as in nature. The simulated light entrainment to rectangular cycles has a similar shape as our calculated entrainment region. The scattering in our results is due to the high precision we required for proper entrainment (approx. 1 min) and the limited entrainment time of 1,000 h. The shape gets smoother with longer entrainment time (not shown). However, we decided to stop our simulation at this point as 1,000 model hours are already quite a long time and entrainment times that are even longer would not be of any physiological relevance.

2.3 Phase response curves

To study the response of our model to temperature pulses, we first entrained our model by 24 h temperature cycles for 234 modeling hours and released it from temperature entrainment at the time point of the lowest temperature. This was regarded to be circadian time 0 (CT0). After further simulation at constant temperature (291 K) for 5 days, temperature pulses were applied at different circadian times. The resulting phase response curves (PRCs) are displayed in Fig. 7a. For the entrainment, we used sinusoidal temperature curves as observed in the natural environment [30] whereas the temperature pulses were simulated with rectangular functions. To determine the circadian time, we used CT of the *c3*-mRNA peak as reference as described by Johnson et al. [1]. For temperature-increase pulses, one expects and observes a phase delay at the beginning of the night and phase advance at the end of the night. During the day, the organisms expect temperature to rise. Therefore, only slight or no responses to temperature-increase pulses in the middle of the day are observed. This is called the dead zone of the PRC and the simulation of this dead zone with mathematical models is known to be difficult [34]. In our simulations, the phase delay during the night is reduced compared to experimental observations [1], but the dead zone is very well simulated. Taken together, our PRC is a typical so-called type 1 PRC and reflects those observed experimentally in other organisms [2].

To investigate the composition of the PRC and to better understand the origin of the dead zone, we restricted the temperature pulse to single reactions to calculate the contribution of each reaction to the observed phase shifts (Fig. 7a). Although the PRCs look different during the night phase, all PRCs display a dead zone during the day. Thus we compared the PRCs to the species concentration time courses. As can be deduced from Fig. 7b the dead zone in the PRC correlates with the time of high concentration of the phosphorylated form of C3.

3 Discussion

The time course of temperature is, of course, an important environmental factor for any living organism. Here, we have analyzed by a simulation study how the circadian rhythm

can be entrained by external temperature and, nevertheless, resist changes to its basic properties upon changes in temperature.

Using experimental data on temperature changes in the green alga *C. reinhardtii*, we built a model of a circadian oscillator that is temperature-compensated and can be entrained by temperature cycles in a certain period range. As experimental data on temperature entrainment and compensation in *C. reinhardtii* are limited and difficult to obtain, we compared our simulation to experimental results from other organisms. Although, the components of circadian clocks differ from organism to organism, there seem to be some general features that are common to all circadian clocks as most of them involve phosphorylation of one or several clock components, complex formation and interlocked feedback loops. Therefore, we believe that the conclusions we draw from our simulation reflect some general features of circadian rhythms.

In the present study, we showed that in our model, the temperature-dependent phosphorylation of the C1 subunit of the RNA-binding complex CHLAMY1 is required for proper temperature entrainment of the oscillator. Entrainment was only achieved with low activation energy for the phosphorylation of C1, which leads to higher phosphorylation at lower temperature, as observed experimentally. In contrast, the temperature compensation of our oscillator is better, with higher activation energies for the C1 phosphorylation, although it is still in an experimentally observed range for high activation energies. Thus, the mechanisms for temperature compensation and entrainment are partially contradictory in our model.

Nevertheless, temperature compensation is required for temperature entrainment in our model, as the uncompensated oscillator cannot be entrained by temperature cycles of any kind. To determine whether this is true in general for circadian oscillators is beyond the scope of this study. However, that temperature compensation might be required to allow entrainment over a broad range of temperatures has already been postulated by Johnson [35]. In addition, a mathematical discussion of this phenomenon can be found in Rand et al. [36].

So far, the kinases and phosphatases involved in the phosphorylation of C1 are not known. In the *per1*-mutant that shows reduced phosphorylation of C1 at both high and low temperature [10], an influence on temperature regulation has been observed. However, the mutated gene is not known so far, although genetic crosses indicate a single locus [37]. As the entrainment is much more sensitive to changes in the phosphorylation reaction compared to the dephosphorylation reaction, the kinase involved in this process especially needs to be identified to be able to modulate the phosphorylation status of C1 experimentally.

Currently, the experimental data on C3-expression are not sufficient to fit the temperature dependent behavior of C3 in our model, and thus we did only attempt to capture the co-regulation of C1 and C3 observed experimentally. We are able to show that the co-regulation is achieved by sequestration of C3 due to complex formation with C1 and does not require any additional feedback. To include temperature and daytime-dependent changes of C3 in our model, we most probably would have to extend the current model. To do so, the temperature-dependent expression level of C3 should be experimentally determined, preferably under constant conditions for several time points, as C3 is known to cycle over the course of a day [17, 18].

To further investigate the entrainment properties of our model, we tested the entrainment to temperature cycles of different amplitude and period, and with different wave forms. We show that our oscillator can be entrained to a certain range of periods, which is dependent on the amplitude of the oscillation. Although our oscillator can in general be entrained by both rectangular and sinusoidal temperature cycles, the range of the entrainment to

rectangular temperature cycles is much more limited. This is in line with an extension of the entrainment limit for gradual lights-on and lights-off transition observed in hamsters [33] and with simulations on light entrainment for *N. crassa* that show that a 12-h transition time, corresponding to a sinusoidal wave forms, has the best entrainment potential [32]. Therefore, our simulations are in good agreement with experimental observations and with previous simulation studies.

As the properties of entrainment are different for rectangular and sinusoidal temperature cycles and as natural temperature variations correspond to sinusoidal temperature curves [30], we suggest to apply if possible sinusoidal temperature cycles in experimental procedures or allow for very slow transition between the temperature levels to avoid possible experimental artifacts.

Finally, we investigated the response of our model to temperature-increase pulses and the resulting PRC is a typical so-called type 1 PRC and reflects those observed experimentally in other organisms [2]. Especially, the dead zone occurring during the day phase was successfully simulated in our calculations.

To better understand the origin of this dead zone and the overall shape of the PRC that is known to be difficult to simulate with mathematical models, we disassembled the PRC by simulating the influence of temperature pulses on single reactions. Although those PRCs are different for the night time, all PRCs contain a dead zone during the day. Thus, we further investigated the species concentration time courses and demonstrate that the dead zone corresponds to times with high C_3^P concentration. This species negatively feeds back on the transcription of $c3$ and thus prevents transcription of $c3$ above a certain concentration threshold. Furthermore, the concentration of C_3^P is far above the K_M value of the corresponding degradation reaction and the degradation is therefore saturated. Taken together, increases in the reaction rates by two-to-three-fold cannot induce large changes in the species time course, due to saturation conditions for C_3^P and the resulting inhibition of transcription, and this seems to be the reason for the observed dead zone in our PRCs.

Unfortunately, we do not have experimental data from *C. reinhardtii* to validate the prediction of our model, but it reflects experimental observation from other organisms and enhances our comprehension of temperature entrainment and PRCs. Although the specific components are different, circadian clock architectures seem to comprise some general features like temperature-regulated phosphorylation and complex formation. Therefore, we believe that the properties and mechanisms for temperature entrainment we identified in our model could be valid for other clock models, too. Thus, our simulations might provide a basis for the theoretical investigation of circadian temperature regulation.

4 Methods

4.1 Mathematical model and simulations

For our simulations, we used the following mathematical model:

$$\frac{dC_3^{mRNA}}{dt} = \frac{v_1}{k_1 + (C_3^P)^2} - \frac{v_2 C_3^{mRNA}}{k_2 + C_3^{mRNA}} \quad (1)$$

$$\frac{dC_3}{dt} = v_3 C_3^{mRNA} - v_5 C_3 - \frac{v_4 C_3}{k_4 + C_3} \quad (2)$$

$$\frac{dC_3^P}{dt} = v_5 C_3 - v_{compl} (C_3^P)^2 C_1^P - \frac{v_6 C_3^P}{k_6 + C_3^P} \quad (3)$$

$$\frac{dC_1}{dt} = v_7 C_1^{mRNA} + v_{dp} C_1^P - v_p C_1 - \frac{v_8 C_1}{k_8 + C_1} \quad (4)$$

$$\frac{dC_1^P}{dt} = v_p C_1 - v_{dp} C_1^P - v_{compl} (C_3^P)^2 C_1^P - \frac{v_9 C_1^P}{k_9 + C_1^P} \quad (5)$$

$$\frac{dCompl}{dt} = v_{compl} (C_3^P)^2 C_1^P - \frac{v_{10} Compl}{k_{10} + Compl}. \quad (6)$$

If not stated otherwise, we applied the following parameter settings:

$$\begin{aligned} v_1 &= 2.6 \text{ nM}^2/\text{h}; k_1 = 0.4 \text{ nM}^2; v_1 = 3 \text{ nM/h}; k_2 = 2 \text{ nM}; v_3 = 5 \text{ h}^{-1}; v_4 = 2.2 \text{ nM/h}; \\ k_4 &= 0.2 \text{ nM}; v_5 = 0.1 \text{ h}^{-1}; v_6 = 1.5 \text{ nM/h}; k_6 = 1.4 \text{ nM}; v_7 = 19 \text{ h}^{-1}; v_8 = 30 \text{ nM/h}; \\ k_8 &= 2 \text{ nM}; v_9 = 1 \text{ nM/h}; k_9 = 1 \text{ nM}; v_{10} = 20 \text{ nM/h}; k_{10} = 4 \text{ nM}; v_p = 1 \text{ h}^{-1}; \\ v_{dp} &= 0.5 \text{ h}^{-1}; v_{compl} = 10 \text{ h}^{-1} \text{ nM}^{-2}; C_1^{mRNA} = 1 \text{ nM} \end{aligned}$$

The initial concentration of all species except C_1^{mRNA} were set to 0. All parameters are assumed to hold for the base temperature $T_B = 291$ K for all calculations. As the existing experimental data are not sufficient to fit the parameters, they were arbitrarily chosen in order to obtain a free-running period of approximately 24 h at 296 K. The model has been submitted to the biomodels database (<http://www.ebi.ac.uk/biomodels-main/>) MODEL1201130000.

For all calculations except PRCs, oscillations were calculated for 1,000 model hours. The phase in PRCs was determined after 520 model hours. The interval size for numerical integration was set to 0.01 h. All calculations except the calculation of control coefficients was done using COPASI version 4.7 [38].

4.2 Computation of period control coefficients

The normalized period control coefficient with respect to the rate constant is defined as [20]:

$$C_i^\tau = \frac{v_i}{\tau} \frac{\partial \tau}{\partial v_i}. \quad (7)$$

To compute the period control coefficients, we follow previous approaches [39–42]. The period control coefficient of the reaction rate constant v_i can be measured by the negative phase shift on the limit cycle after one period τ , if the perturbation starts on the limit-cycle $\mathbf{X}^0(t)$ [40, 41]:

$$\frac{\partial \tau}{\partial v_i} = -\frac{\partial \phi}{\partial v_i}(\tau) = -\sum_{j=1}^n \frac{\partial \phi}{\partial X_j}(\tau) \frac{\partial X_j}{\partial v_i}(\tau) = -\mathbf{Q}(\tau) \cdot \frac{\partial \mathbf{X}}{\partial v_i}(\tau). \quad (8)$$

The function $Q_j(t)$ is known as the infinitesimal PRC of the system with respect to a small perturbation in the concentration of the model species over time ($X_j(t)$) and can be computed by integrating backwards in time $t \leq t_f$ the ODE-system by Ermentrout and Koppel [42]:

$$\frac{d\mathbf{Q}(t)}{dt} = -\mathbf{J}(t)^T \mathbf{Q}(t). \quad (9)$$

Here, $\mathbf{J}(t) = \partial \mathbf{F} / \partial \mathbf{X}|_{\mathbf{X}=\mathbf{X}^0(t)}$ is the Jacobian matrix evaluated on the limit cycle and the final condition is $\mathbf{Q}(t_f) = (0, \dots, 0, 1/F_j(\mathbf{X}^0(t_f)), 0, \dots, 0)^T$ with $\mathbf{F}$ being the right-hand side of our ODE system. Finally, the sensitivity coefficients of the model species on the limit cycle $\mathbf{X}^0(t)$ with respect to changes in the reaction rate constant v_i , $\partial \mathbf{X}(t) / \partial v_i$, are computed by the standard Green's function method [39].

Calculations were done using MATLAB.

4.3 Modeling temperature changes

The temperature dependency of reaction rates v_i was simulated using the Arrhenius equation:

$$v = v_b e^{\frac{E_A}{R} \left(\frac{T_B - T_A}{T_B T_A} \right)}, \quad (10)$$

where v_b corresponds to the reaction rates and maximal velocities at base temperature in the model description. For E_A , different values were applied. C1 translation and degradation as well as C_1^P dephosphorylation and degradation and complex degradation were simulated using the standard activation energy of $E_A^S = 67$ kJ/mol. For the reactions of the negative feedback loop and the complex formation, $E_A^{high} = 84$ kJ/mol was applied to rates with positive control coefficients and $E_A^{low} = 50$ kJ/mol was applied to rates with negative control coefficients. The phosphorylation of C1 was modeled with an activation energy of $E_A^p = 40$ kJ/mol if not stated otherwise.

4.4 Simulation of temperature entrainment

If not stated otherwise, temperature entrainment was modeled using the following equation for sinusoidal temperature cycles:

$$T_A = 296\text{K} + \frac{A}{2} \sin \frac{2\pi t}{\tau'} \quad (11)$$

with A and τ' being the amplitude and the period of the temperature cycles, respectively.

For temperature entrainment with rectangular temperature cycles, the temperature was set to $296\text{K} + \frac{A}{2}$ for day and $296\text{K} - \frac{A}{2}$ for night. The lengths of day and night are both equal to $\frac{\tau'}{2}$.

Entrainment was evaluated after 1,000 model hours by calculating the time difference between the last three c3-mRNA peaks. If the difference was equal to $\tau' \pm 0.02$ h, successful entrainment was assumed. If this was not the case, period doubling was assumed if the sum of the differences was equal to $2\tau' \pm 0.06$ h. In this case the length of each cycle alternates around the period of the temperature cycle. In all other cases, we assumed that entrainment is not possible.

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