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Hybrid models for the interconnection of circadian and genetic cycles in cyanobacteria*

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EXTENDED ABSTRACT

I. INTRODUCTION

This paper will discuss the coupling of two oscillators in cyanobacteria. The circadian rhythm of cyanobacteria is driven by an oscillator based on an ordered sequence of phosphorylations of a protein (KaiC) [13], [14], [18]. As the cyanobacterial cell grows and divides, the amount of protein KaiC itself is regulated by a transcription/translation cycle [16], [22]. The circuitry underlying the cell cycle of cyanobacteria is very poorly known; recently, single cell experiments confirmed the reality of the phenomenon of circadian gating of cell division: there is a phase of the circadian oscillation during which cells divide less frequently [4], [6]. On the other hand, under different periodic light regimes, colonies of cyanobacteria show cell division events taking place at specific phases [5].

Circadian oscillations of cyanobacteria have been shown to be extremely resilient with a measured correlation of several months under constant environmental conditions [12]. To elucidate how it can achieve such remarkable accuracy in spite of the noisy intracellular environment is the main goal of our study.

Our goals are to study: (i) the robustness of the circadian rhythm with respect to the perturbations inherent to the noisy environment of the cell, including cell growth and division; and (ii) to what extent the growth curves of cyanobacterial colonies, under different external light conditions, are modulated by the circadian cycle.

In order to do so, we will study the coupling between the phosphorylation and transcription/translation cycles underlying the circadian clock. Furthermore, based on the results from recent experiments, we will study a simplified model of the cell division cycle including a coupling that permits the circadian clock to modulate its dynamics.

A combination of hybrid and continuous approaches [8], [2] will be used to identify basic mechanisms and analyse the dynamics, according to the following steps. Discrete or Boolean models will be used to identify the logical structure of the interconnection between cycles [21]. Then for the analysis of the dynamics of the parts and of the whole system, we will use a combination of techniques ranging from piecewise affine systems to a (fully) continuous ODE model; eventually including stochastic perturbations arising from the kinetics of chemical reactions and the random partitioning of molecules upon cell division.

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II. THE CORE CIRCADIAN OSCILLATOR

In previous work, we have developed qualitative and quantitative models [3] of the KaiC phosphorylation cycle, based on the data available in the literature from *in vitro* experiments [18], in which Kai(A,B,C) proteins were isolated together with ATP in a test tube. The phosphorylation cycle follows the scheme shown in Fig. 1 (main box), with the variables x_A and x_U representing the concentration of free proteins KaiA and unphosphorylated KaiC, respectively; and x_j, with $j = T, TS, S$, representing the three different phosphorylation states of KaiC. The quantitative model is:

$$\begin{aligned} \dot{x}_A &= k_A h^-(x_S, \theta_S, n) - \gamma_A x_A \\ \dot{x}_T &= k_T h^+(x_U, \theta_U, n) h^+(x_A, \theta_A, n) - \gamma_T x_T \\ \dot{x}_{TS} &= k_{TS} h^+(x_T, \theta_T, n) h^+(x_A, \theta_A, n) - \gamma_{TS} x_{TS} \\ \dot{x}_S &= k_S h^+(x_{TS}, \theta_{TS}, n) h^-(x_A, \theta_{A,2}, n) - \gamma_S x_S \\ x_U &= x_{Tot} - x_T - x_{TS} - x_S, \end{aligned} \tag{1}$$

where the last equation stands for the conservation of the total number of KaiC molecules in the test tube. The parameters of the system are shown in Table I; these were estimated by fitting the system's evolution to experimental data. The functions h^+ and h^- represent increasing and decreasing Hill functions:

$$h^+(x, \theta, n) = \frac{x^n}{x^n + \theta^n}, \quad h^-(x, \theta, n) = \frac{\theta^n}{x^n + \theta^n},$$

where n represents the Hill coefficient. This model has a periodic orbit with frequency of 24.1 hours, and reproduces the experimental properties observed in [18], [14], [11], [17]. In the limit $n \to +\infty$, model (1) reduces to a piecewise affine system, where the Hill functions are replaced by step functions:

$$s^+(x, \theta) = \begin{cases} 1, & x > \theta \\ 0, & x < \theta, \end{cases}, \quad s^-(x, \theta) = 1 - s^+(x, \theta).$$

The advantage of such a system is that it is more amenable to an analytical study [1]; several theoretical results were proved in [3] concerning its asymptotic behavior.

III. THE CELL DIVISION CYCLE

Despite the scarcity of molecular data on the genetic mechanisms of both the circadian clock and the cell division cycle in cyanobacteria, it is clear that these are closely connected with the phosphorylation cycle [16], [22], [19].

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TABLE I

PARAMETERS FOR MODEL (1), AS ESTIMATED AND JUSTIFIED IN [3].

Parameter	Value
n	4
k_A	10
k_T	20.51
k_{TS}	10.74
k_S	6.61
γ_A	0.45
γ_T	0.24
γ_{TS}	0.28
γ_S	0.0817
θ_A	10.0
$\theta_{A,2}$	$1.3\theta_A$
θ_T	11.42
θ_{TS}	10.16
θ_S	5.0
θ_U	29.95

In this paper we study the coupling of the KaiC circadian oscillator with a simplified model of the cell division cycle to assess and test the robustness of the circadian rhythm with respect to the perturbations inherent to the noisy environment of the cell. For this purpose, we will thus consider that the isolated system (1) is now inside the cell. Protein KaiC will now be created from the transcription and translation of the corresponding gene; this transcritption being under the negative feedback control by the KaiC protein in its different phosphorylation states. Cell growth affects the dynamics via a linear dilution μ term related to the average time for cell division. [1]

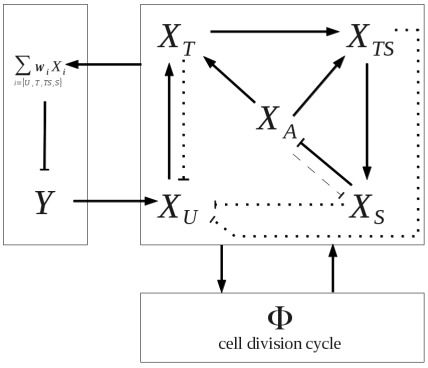

Fig. 1. The KaiC phosphorylation cycle (main box) interacts with gene transcription (y =KaiC mRNA) and the cell division. The transcription is regulated by a linear conbination of all the configurations of protein KaiC. The dotted lines represent a conservation law, while the dashed line represents a weaker effect when compared to the solid lines.

A. Modeling transcription and translation

The messenger RNA for protein KaiC will be denoted y, and its transcription will be downregulated by a combination of the various configurations of protein KaiC (see also Fig. 1), as follows:

$$\begin{aligned} \dot{y} &= k_y h^-(x_c, \theta_c, n) - \gamma_y y \\ x_c &= w_U x_u + w_T x_T + w_{TS} x_{TS} + w_S x_S. \end{aligned} \tag{2}$$

Inside the cell, the total amount of protein KaiC is not constant; the synthesis of its unphosphorylated configuration from gene transcription and translation will be modeled by a term of the form $k_U h^+(y, \theta_y, n)$). In addition, the concentration of all proteins will be affected by cell growth, which adds a linear dilution term with constant μ, for simplicity. The total amount of protein KaiC will now satisfy an equation of the form:

$$\begin{aligned} \dot{x}_{Tot} &= \dot{x}_U + \dot{x}_T + \dot{x}_{TS} + \dot{x}_S \\ &= k_U h^+(y, \theta_y, n) - \mu(x_U + x_T + x_{TS} + x_S). \end{aligned}$$

By rewriting this as a differential equation for x_U and adding the dilution terms to each equation, the interconnected circadian/genetic system can be written:

$$\begin{aligned} \dot{x}_A &= k_A h^-(x_S, \theta_S, n) - \tilde{\gamma}_A x_A \\ \dot{x}_T &= k_T h^+(x_U, \theta_U, n) h^+(x_A, \theta_A, n) - \tilde{\gamma}_T x_T \\ \dot{x}_{TS} &= k_{TS} h^+(x_T, \theta_T, n) h^+(x_A, \theta_A, n) - \tilde{\gamma}_{TS} x_{TS} \\ \dot{x}_S &= k_S h^+(x_{TS}, \theta_{TS}, n) h^-(x_A, \theta_{A,2}, n) - \tilde{\gamma}_S x_S \\ \dot{x}_U &= k_U h^+(y, \theta_y, n) - f(x_A, x_T, x_{TS}, x_S) - \mu x_U \end{aligned} \tag{3}$$

where

$$\tilde{\gamma}_r = \gamma_r + \mu, \quad r \in \{A, T, TS, S\},$$

and

$$\begin{aligned} &f(x_A, x_T, x_{TS}, x_S) = \\ &\ k_T h^+(x_U, \theta_U, n) h^+(x_A, \theta_A, n) - \gamma_T x_T \\ &+ k_{TS} h^+(x_T, \theta_T, n) h^+(x_A, \theta_A, n) - \gamma_{TS} x_{TS} \\ &+ k_S h^+(x_{TS}, \theta_{TS}, n) h^-(x_A, \theta_{A,2}, n) - \gamma_S x_S, \end{aligned}$$

with y given by (2).

The new parameters are given in Table II. The value of $\mu = \ln 2/T$ was calculated based on the assumption that the cell divides, on average, every T hours. The values for k_U, k_y, γ_y, and θ_y were arbitrarily chosen but with the same order of magnitude as the k_i, γ_i, or θ_i in Table I. The weights w_j are based on the paper [], where the form KaiC-T is observed to play a small role.

B. Modeling cell division

At cell division, the "mother" cell splits into two "daughter" cells, implying a partition of the molecules between its offspring. In an "ideal" division, the total number of molecules of each species would be equally divided between the two daughter cells, their volumes exactly halved with respect to that of their progenitor, and the dynamical evolution of equations (3) would proceed unperturbed. However, in practice, this molecular partition is subject to random fluctuations which often follow a binomial distribution [9].

We wish to study the effect of these intrinsic fluctuations in the circadian clock. To do this, we need to: (i) specify

[1] We will assume that the parameters in Table I estimated for the isolated system in a test tube are still valid, which may be a strong assumption, but the effect of varying these parameters will be considered.

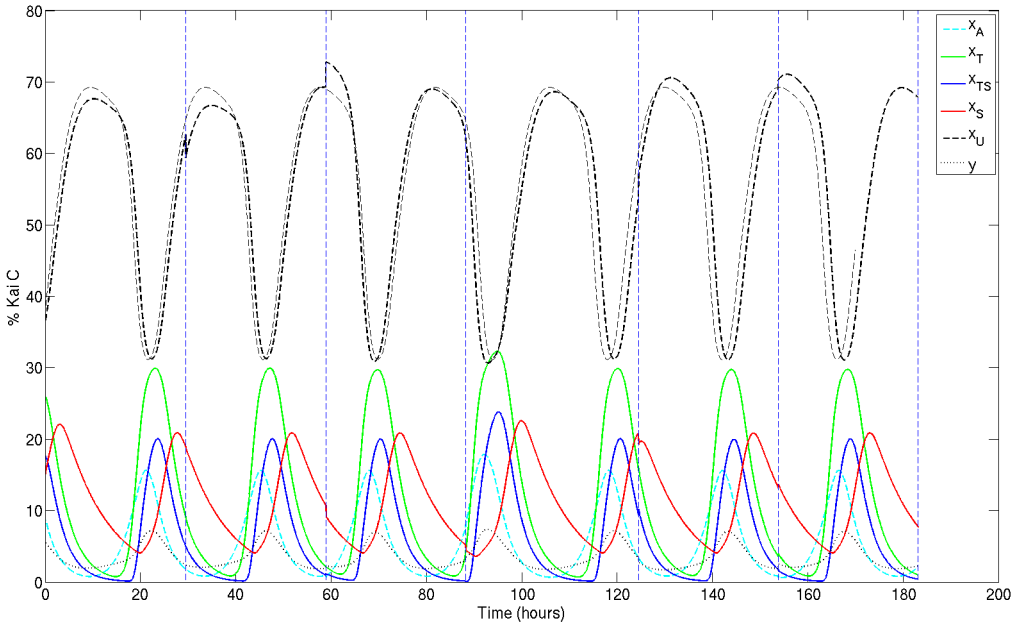

Fig. 2. The solutions of system (3)-(4). The curves are as indicated in the legend. For comparison, the thin black dashed curve corresponds to the "ideal" system, where partition of molecules between the two daughter cells is equal. The vertical dashed blue lines represent the instants at which cell division occurred.

TABLE II
ADDITIONAL PARAMETERS FOR MODEL (3)-(4) (THE JUSTIFICATION OF THESE VALUES IS GIVEN IN THE TEXT).

Parameter	Value
k_U	10
k_y	10
γ_y	0.4
θ_y	5
θ_c	$w_U\theta_U + w_T\theta_T + w_{TS}\theta_{TS} + w_S\theta_S$
w_U	1
w_T	0
w_{TS}	1
w_S	1
μ	$(\ln 2)/24$
n_ϕ	8
k_ϕ	$2\pi/24$

how and when cell division takes place; and (ii) generate the random fluctuactions in the concentrations of all molecules present at these events. This is done by modelling the cell cycle by a phase oscillator of the form

$$\dot{\phi} = k_\phi h^-(x_A, \theta_A, n_\phi), \qquad (4)$$
$$\text{if } \mod(\phi, 2\pi) = 0, \text{ then cell division occurs,}$$

where k_ϕ is the free-running frequency and the coupling with the circadian oscillator is represented by a negative Hill function h^-. Every time the phase crosses $\phi = 2\pi$ cell division takes place, and molecules are randomly partitioned between the offspring. Cyanobacteria are known to grow and divide at different rates depending on the level of irradiance to which they are exposed, so k_ϕ is a parameter that can be varied.

The experiments performed by [4] have highlighted a strong correlation between the gating of cell division and peaks in ATPase activity. We do not include this ATPase activity in our model; therefore, for us the crucial fact is that the latter peak happens roughly 4 hours before the phosphorylation peak [20], and coincides with the free KaiA peak in our model. We therefore introduce the coupling $h(x_A, \theta_A, n_\phi)$ centered on the peak of free KaiA in such a way that cell growth slows down; cell division events are significantly suppressed during that period, as can be seen in Fig. 2.

C. Further model analysis

To complement model (2)-(4) and improve our understanding of the Kai(A,B,C) systems, several steps will be performed.

First, the parameters in Table II will be estimated more precisely and different weight combinations (w_i) will be tested and compared.

Second, in order to quantify the robustness with respect to cell division, we will measure the phase shift of the system as time evolves. The period of the cell cycle phase (which is given through parameter k_ϕ) will be varied and its effect on the circadian rhythm will be measured.

Third, a fully stochastic approach that takes into account the effect of small fluctuations inherent to chemical kinetics and random partitioning due to cell division will be implemented and the results compared to those of the continuous, deterministic model (2)-(4). We will employ a Gillespie type algorithm [7], [10].

IV. Results and discussion

Simulations show that model (2)-(3)-(4) has a periodic solution with period close to 24 hours. Cell division events introduce small jumps in the concentrations as illustrated in Fig. 2. Comparison of the free KaiC protein (x_U) for the unperturbed (thin black dashed curve) and perturbed (bold black dashed curve) systems shows that a phase shift is setting in. The result is that the phase will undergo a random walk and slow phase drift will become evident at long times. Preliminary results suggest that the expected correlation time is of order of hundred days, a result which would be compatible with observations [12].

This modeling approach, which couples a continuous or piecewise affine model with discrete events (here, cell division and random partitioning) remains very intuitive; it has obtained pertinent results both at the theoretical level [3] and of the comparison to experimental data succeeding in reproducing experimental observations.

This approach is suitable to study problems concerning the robustness of biological oscillators, including when they are coupled with other modules, whether autonomously oscillating or not. Several aspects to be analyzed in this work include the response of the KaiC phosphorylation cycle to fluctuations arising from cell division events and from the stochasticity inherent to chemical kinetics, and how that response impacts its phase re-adjustments and the robustness of its phase-tracking mechanism—or, in other words, its capacity accurately keep track of the passage of time.

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