

Coupling cellular oscillators—circadian and cell division cycles in cyanobacteria

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Understanding how different cellular subsystems are coupled to each other is a fundamental question in the quest for reliably predicting the dynamic state of a cell. Coupling of oscillatory subsystems is especially interesting as dynamic interactions play an important role in cell physiology. Here we review recent efforts that investigate and quantify the coupling between the circadian and cell cycle clocks in cyanobacteria as a model system. We discuss studies that quantify the coupling from a systems point of view in which the oscillators are described in abstract terms. We also emphasize recent developments aimed at uncovering the molecular details underlying the coupling between these systems. Finally we review recent studies that describe a potentially more overarching regulation scheme through global circadian regulation of DNA packing and gene expression.

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Introduction

Oscillatory processes spanning a wide range of timescales play a central role in a variety of biological systems: neural spike trains, glycolytic cycles, periodic cell division, DNA replication, respiratory and cardiac activity in vertebrates, circadian oscillations and reproduction cycles in plants and animals, to name a few. In natural contexts these cyclic processes often interact with other subsystems. For instance, external environmental cues could affect the frequency of oscillators or reset their state; some clocks could interact with other oscillators acting as pacemakers and synchronizing the dynamical state of a tissue consisting of a population of cells; alternatively they could just affect the dynamics of downstream processes. Therefore, investigating how different biological clocks interact with each other and with other cellular subsystems

becomes an important question that allows one to better understand and predict the dynamical state of cells.

From a systems point of view one could abstract the dynamics of simple oscillators using phase variables [1,2]. These variables quantify which part of the cycle the system is going through and interactions with other systems (oscillatory or not) can be described as inputs that regulate the progression of these phases. This approach allows one to rationalize the behavior on a macroscopic scale and to test whether a system could be thought of as a periodic oscillator interacting with other entities or whether the notion of a semi-autonomous oscillator breaks down due to the complexities of interactions with other agents. In this context identifying the properties of the coupling with other systems becomes one of the most interesting questions. When does one oscillator affect other processes? When is an oscillator affected by other processes? How much are its free-running dynamics changed by such interactions? Complementary to answering these systems-level questions is the elucidation of the molecular bases that underlie the oscillatory phenomenon and reveal the molecular mechanism responsible for its coupling with other subsystems.

The circadian oscillator in cyanobacteria

The cyanobacterium *Synechococcus elongatus* PCC7942, a unicellular organism that exhibits circadian oscillations [3–6], has become a powerful model system to study some of these questions. Circadian oscillators are autonomous, temperature-compensated systems that cycle with a period of approximately 24 h under constant environmental conditions and whose activity can be reset with external cues like alternating periods of light and dark. These types of oscillators are believed to have evolved in adaptation to the daily cycles of our planet and are ubiquitous across many species [7].

The ability to modify *S. elongatus* genetically [8] provided the flexibility required for testing mechanistic hypotheses about the regulation of several of its intracellular systems and has led to the development of bioluminescent [9,10] and fluorescent reporter systems [11] that allowed researchers to quantitatively track the state of the circadian clock in single cells. During the past 20 years significant work has been devoted to elucidating the molecular basis of this circadian oscillator and it is now believed that it consists, at its core, of three proteins, *KaiA*, *KaiB* and *KaiC*, that dynamically regulate the phosphorylation state of the latter protein [12,13] in a

transcription independent manner [14,15]. These significant developments allowed researchers to start thinking about how this oscillator interacts with other cellular subsystems, and especially with other periodic processes.

The fact that circadian oscillations are present in *S. elongatus* single cells allows one to decouple the contribution and potential synchronization effects coming from neighboring cells in tissue-based models and makes the organism specially suited for the study of how the circadian system couples with other cellular subsystems, both from a systems and molecular point of view.

Coupling between the circadian oscillator and the cell division cycle

In 1996, Mori *et al.* [16] reported observations that the circadian clock in *S. elongatus* is not only a robust oscillator (even when cells divide about twice per day [10,17,18]), but also that it regulates cell division by ‘circadian gating’. After synchronizing a population of cells with 12 h cycles of light and dark, the population was grown in an environment of constant light. Surprisingly it was observed that the rate of cell divisions is modulated with a 24 h period as well, with less divisions taking place at specific circadian times. Using flowcytometry it was found that cell size exhibits a similar periodicity, which suggested that the size of the cells is not uniform throughout the day. By quantifying the average amount of DNA content per cell the rate of DNA synthesis could be estimated, and it was uncovered that this rate remains approximately constant throughout the circadian cycle. These experiments suggest a connection between the circadian oscillator and other cyclic systems in this organism: the cell division cycle is affected by the circadian system but the cyclic synthesis of DNA is not. Interestingly, this indicates that in this organism there is no mechanism to synchronize DNA replication and cell division. Once the fact that there is some coupling between these two systems was established the question became how to quantify it and to discover how it is implemented at the molecular level.

Systems-level description of the coupling

In a recent study, Yang *et al.* [19] studied the coupling between the circadian and cell division oscillators using time-lapse microscopy [20], tracking both the state of the circadian oscillator by monitoring YFP expression driven by the *kaiBC* promoter, and cell division events as identified through image analysis. This study uses phase variables to describe the state of the circadian and cell division cycles. This dynamical information was used to quantify the coupling between the two oscillators by considering the effect of the circadian state on the velocity of progression through the cell division cycle using a mathematical model, based on ideas pioneered by Winfree [1], Strogatz [2] and Glass [21] among others.

This study simplifies the representation of the dynamics of the underlying processes to two differential equations that describe how the phases of the circadian (θ) and cell cycle (ϕ) clocks change with respect to time.

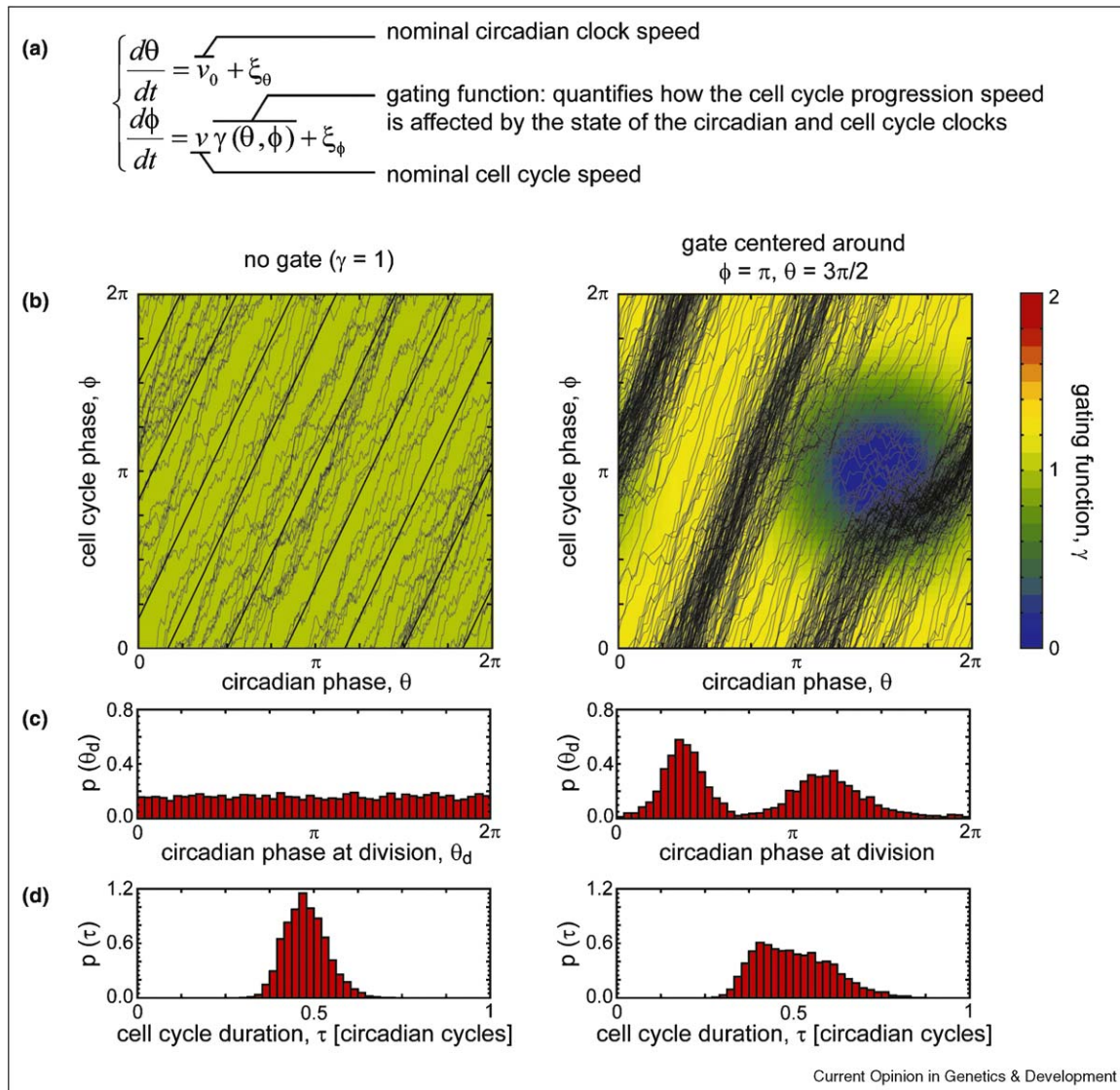
$$\begin{cases} \frac{d\theta}{dt} = \nu_0 + \xi_\theta \\ \frac{d\phi}{dt} = \nu\gamma(\theta, \phi) + \xi_\phi \end{cases}$$

These phases are periodic variables normalized to fall in the $[0, 2\pi]$ range. In the equations governing the dynamics of these variables, ν_0 is the speed of the circadian clock, ν describes the average speed of cell cycle progression and $\gamma(\theta, \phi)$, the coupling, is a non-negative function describing how the state of the two clocks affects cell cycle progression. The terms ξ_θ and ξ_ϕ are white noise terms representative of intrinsic fluctuations. In the absence of any coupling ($\gamma(\theta, \phi) = 1$) the two phases evolve independently of each other with average speeds ν_0 and ν respectively. If the coupling function is not uniform, the progression through the cell cycle will depend on the phases of the two oscillators. In other words, depending on the state of the system the cell cycle will progress either more slowly or more rapidly than average: cell cycle progression will be gated by the clocks and this will lead to different distributions of cell divisions throughout the day compared with the case in which there is no gating (Figure 1). Likewise, the distribution of cell cycle durations is also affected by this phenomenon.

This theoretical framework allows one to contrast the observed distribution of individual cell divisions throughout the day, as well as the duration of individual cell cycles, with the results derived from different gating functions. By searching for the gating function that best described the experimental data it was suggested that the extent of circadian gating in this organism is consistent with an overall down regulation of cell cycle progression during a window of approximately 6 h centered around 17 h circadian time (0 corresponding to the onset of light). This work also showed that the coupling is relatively independent of the average speed of the underlying cell division process: the same coupling function was able to describe the behavior of population of cells that grew with different average cell division rates. This was achieved by considering experiments performed under different intensities of environmental light, which directly affects the rate of cell duplication.

These observations suggest a simple abstract model in which the speed of progression through the cell division cycle is essentially slowed down during the subjective circadian night, but the study does not dig into the complementary problem of how the ‘gating’ mechanism is implemented at the molecular level.

Figure 1



Gating model in terms of cell cycle phase speed regulation. **(a)** Mathematical formulation of the model proposed in [19*] (see text). **(b)–(d)** Monte Carlo simulations of the model for situations in which there is no gating (left) or a narrow gate centered around cell cycle phase $\phi = \pi$ and circadian phase $\theta = 3\pi/2$ (right). In both cases the nominal speed of the cell cycle clock is $\nu = 2.1\nu_0$ and the strength of the noise terms used is $\xi_{\theta} = 0, \xi_{\phi} = 0.1\nu_0$. **(b)** Color-coded coupling function and steady-state organization of trajectories in (ϕ, θ) space. In the no-gate case, straight lines show the deterministic behavior. In the situation depicted on the right cell cycle progression slows down around the gate, that is where γ is close to zero. **(c)** Steady-state distribution of circadian phases at which divisions take place. In the case of no-gate divisions are uniformly distributed throughout the day (left). With a non-trivial gate there are portions of the day at which the number of cell divisions is diminished. **(d)** Steady-state distribution of cell cycle durations. In the case in which there is a gate the distribution of durations broadens.

Molecular basis of circadian ‘gating’

In 2005, Miyagishima *et al.* [22] reported a transposon mutagenesis analysis in which they probed cyanobacteria for genes related to cell division. Interestingly, they found the *CikA* (circadian input kinase) gene among candidates, providing a first molecular link between the circadian cycle and cell division processes. A *cikA* null mutant

exhibits an elongated phenotype, which indicates some misregulation of normal cell division behavior. This gene, however, is thought to be part of what is considered the input of the circadian oscillator. Schmitz *et al.* [23] showed that it is a main player in relaying environmental cues to the circadian circuit and that it plays a fundamental role in the ability of the system to synchronize to external light–

dark cycles. The question of how the cell division machinery is affected by this player was still open.

Following up on this work, Mackey *et al.* [24[•]] reported a screen based on the yeast two-hybrid assay, with which they identified the gene *CdpA* that interacts with *CikA* and has an effect on the elongated phenotype of *cikA* null mutants: overexpressing *CdpA* in this background with an IPTG-inducible system leads to recovery of wild-type like cell lengths.

Recently, Dong *et al.* [25[•]] attacked this question using a combination of molecular biology and genetics complemented with single cell microscopy. By considering the effect on average cell length of single or double deletions of different genes as well as different phosphorylation mutants of *KaiC* the researchers were able to show that the ATPase activity of *KaiC* is linked to inhibition of cell division. It was observed that *CikA* has an effect on cell length, but the observations are consistent with it being contingent on the ATPase activity of *KaiC*. They further show how disruption of *SasA* or *RpaA* interferes with circadian-regulated cell division downstream of the *kaiABC* genes, suggesting that they are part of the signaling system that relays the circadian signal to the cell division machinery. *SasA* is a histidine protein kinase that is known to phosphorylate *RpaA*, a putative transcription factor. Finally, they connected the link with cell division by using immunofluorescence microscopy and observed the mislocalization of the midcell *FstZ* ring in strains in which the proposed pathway had been disrupted. *FstZ* is a conserved bacterial homolog of tubulin that is usually expressed in a ring in the middle of a cell just before it divides. Though there are probably more intermediate steps between the identified players and perhaps some other pathways might regulate other stages of the cell division cycle, this study identified some of these mechanisms, leading to a better understanding of how these two clocks are coupled to each other.

But the aforementioned mechanism is not the only process that is known to be regulated by the circadian system in *S. elongatus*. In this organism the circadian clock has been shown to have an overarching impact on gene expression [26,27]: when the *luxAB* luciferase gene is inserted downstream of the promoter of essentially any gene, circadian bioluminescent signals are observed, implying that gene expression patterns are periodically affected by the state of the circadian clock. This phenomenon was shown to be dependent on *SasA* [28]. The differential role of the sigma factors *RpoD2*, *RpoD3*, *RpoD4* and *SigC* on the periodic expression of some genes was revealed a few years ago [29].

Recently, Smith and Williams, using fluorescence microscopy on DAPI-stained cells, showed that circadian rhythms in cyanobacteria also regulate chromosome com-

paction [30] in a *SasA*-independent manner. In a 2009 study [31[•]] researchers reported that, in general, expression levels in cyanobacteria are correlated with the supercoiling state of DNA. They show that when inducing a transition from a supercoiled to a relaxed state (by treating cells with the gyrase inhibitor novobiocin) expression patterns followed the trajectory predicted by the previously observed correlation. These observations suggest some other potential indirect pathways through which the circadian clock might regulate cell division.

Coupling between circadian and cell cycle oscillators in other systems

The gating of cell division by the circadian clock has been observed in other organisms as well, especially in mammals, and researchers have been investigating how the coupling is implemented in those systems.

Matsuo *et al.* [32] observed circadian gating of cell divisions in the regenerating liver of mice and proved that the gene *Wee1* plays a central role in the coupling process. They hypothesize that in their system circadian genes control transcription of *Wee1*, which then modulates the expression of key mitosis regulators such as the Cyclin *B1-Cdc2* kinase.

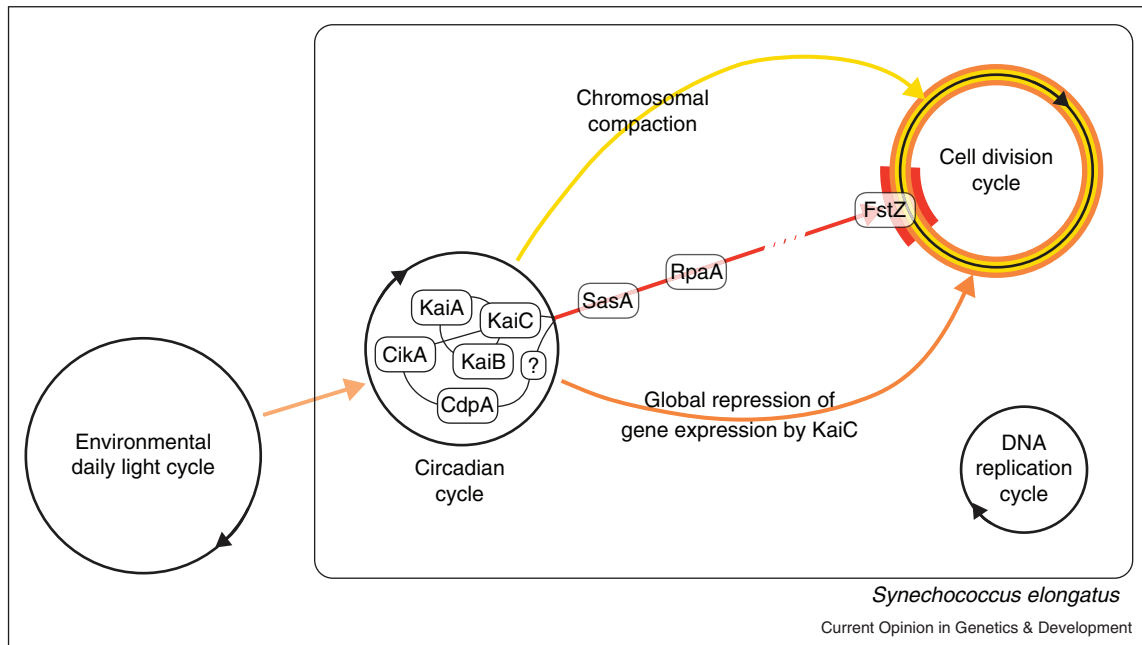
In zebrafish, Dekens *et al.* [33] reported the synchronization of cell cycle progression after exposure of larvae to cycles of light and dark and the propagation of such synchronization for several days after transitioning to constant light environments.

In individual mouse fibroblasts, Nagoshi *et al.* [34] investigated whether circadian oscillations were maintained after detachment from circadian pacemakers in the suprachiasmatic nucleus. They found not only that individual cells are able to maintain autonomous circadian oscillations but also that the timing of cytokinesis is gated by the state of the circadian clock. Interestingly, in their experiments they observed that cell divisions in this system are distributed throughout circadian time following a structure with reduced number of divisions in 3 episodes throughout the cycle. For a simple effective gating mechanism like that observed in cyanobacteria this would be possible only in the case in which the cells divide about 3 times faster than the circadian clock, which opens the possibility that the coupling scheme in this system is of a more complicated nature.

Conclusions

The cyanobacterium *S. elongatus* has been proven to be a powerful model system to study the coupling between different cellular oscillators. In this system the coupling works essentially in one direction: the robust circadian system regulates the cell division cycle. Researchers have been hunting for the mechanisms that underlie this interaction, having identified important roles of proteins

Figure 2



Interactions between clocks in cyanobacteria. The diagram represents some of the observed interactions between different biological clocks in the cyanobacteria *Synechococcus elongatus*. Each clock is shown as a circular arrow. The daily natural cycles of light and dark interact with the intrinsic circadian oscillator through CikA. This protein interacts with KaiC, one of the circadian clock main players (the others being KaiA and KaiB), which then, through its ATPase activity, affects SasA activity at a specific circadian phase, leading to a chain of interactions that end up in misregulation of FstZ localization and disruption of a phase of the cell division clock. The discovered interaction of CikA with CdpA is also shown, as it presents a potential additional mechanism for the coupling between these two clocks. Two other potential mechanisms by which the cell division cycle could be affected at a specific circadian phase are shown: overall downregulation of gene expression and chromosomal supercoiling. Finally, DNA replication is shown isolated as evidence suggests that its dynamics are independent of the activity of the others.

like CikA, SasA, RpaA and FtsZ as well as more overarching schemes like chromosomal compaction and overall downregulation of gene expression during some parts of the circadian cycle (Figure 2).

However, the situation in which the coupling is essentially unidirectional is not necessarily the only possibility when one considers the idea of two interacting oscillatory systems. Systems in which both clocks affect each other is potentially richer from the dynamical point of view and it would be interesting to see whether and how such situation arises in the context of cellular oscillators.

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