Minireview

Global orchestration of gene expression by the biological clock of cyanobacteria

Carl Hirschie Johnson

Address: Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA. E-mail: carl.h.johnson@vanderbilt.edu

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Abstract

Prokaryotic cyanobacteria express robust circadian (daily) rhythms under the control of a central clock. Recent studies shed light on the mechanisms governing circadian rhythms in cyanobacteria and highlight key differences between prokaryotic and eukaryotic clocks.

Rhythmic gene-expression patterns

Circadian biological clocks are self-sustained biochemical oscillators. Their properties include an intrinsic time constant of approximately 24 hours, temperature compensation (so that they run at a period of 24 hours irrespective of temperature), and entrainment to daily environmental cycles [1]. Many biological processes are controlled by these clocks, including gene expression, neuronal activity, photosynthesis, sleeping and waking, and development. Microarray analyses of mRNA expression patterns in eukaryotes have demonstrated that 5-10% of genes exhibit daily rhythms of mRNA abundance. But mRNA abundance is not necessarily comparable with transcriptional activity. For example, microarray and promoter-trap experiments in the eukaryote *Arabidopsis* have demonstrated that only 6% of genes showed rhythms of mRNA abundance [2], whereas about 35% of promoters were rhythmically controlled [3]. These results imply that the promoters of many eukaryotic genes are controlled by the biological clock, but that post-transcriptional control mechanisms counterbalance the rhythmic transcriptional activity of some genes so that their mRNA abundances are constant.

In prokaryotic cyanobacteria, it is not a mere fraction of the total entourage of promoters that is regulated by the daily biological clock; rather, there is global control of promoter activity by the daily timekeeper [4]. This remarkable property was demonstrated by a promoter-trap experiment using random insertion of a promoterless luciferase gene throughout the genome of *Synechococcus elongatus*. Of the more than 800 insertion-line colonies analyzed, all displayed circadian rhythms of glowing luciferase function with the same period [4]. The pattern of rhythmic expression differed between the promoters, in terms of both phasing and waveform (Figure 1a-d). Heterologous promoters, such as an *Escherichia coli* promoter (*conII*p) were also transcribed rhythmically when inserted into the cyanobacterial chromosome [5]. Apparently the cyanobacterial clock controls gene expression globally - by regulating the activity of all promoters. No microarray analysis of mRNA abundances in cyanobacteria has yet been reported, but it is likely - as in the case of *Arabidopsis* - that some of the genes whose promoter activities are rhythmic may exhibit 'de-regulated', that is non-rhythmic, patterns of mRNA abundance.

KaiC, a master regulator of rhythmic gene expression

In cyanobacteria, there are at least three essential clock-specific genes, *kaiA, kaiB*, and *kaiC*, that form a cluster on the chromosome [6]. Some features of *kai* gene regulation appear reminiscent of the regulation of eukaryotic clock genes. For example, there are rhythms in the abundance of the *kaiA* and *kaiBC* transcripts [6] and of the KaiB and KaiC proteins [7,8]. KaiA, KaiB and KaiC interact with each other [9,10] and with a histidine kinase, SasA [11]; these interactions appear to lead to the formation of protein complexes *in vivo* [12]. KaiC exists in phosphorylated forms *in vivo* [8], suggesting another similarity to the post-translational control of eukaryotic clock proteins.

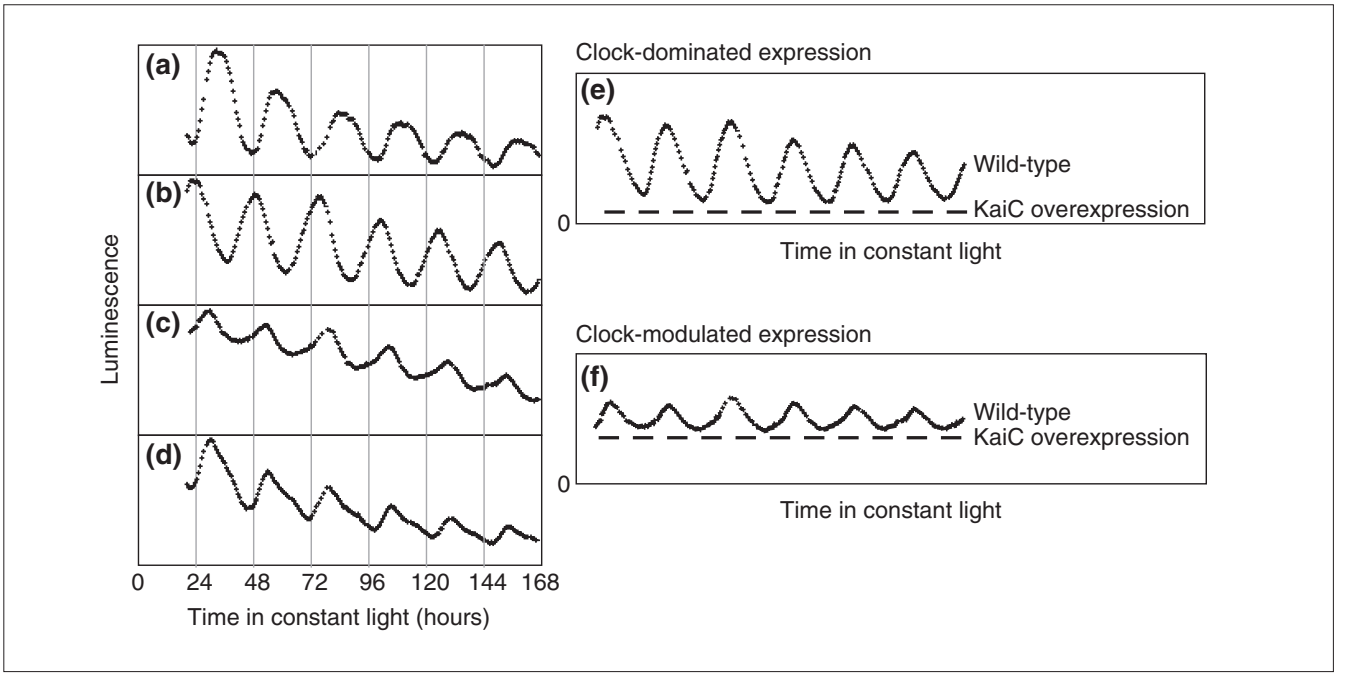

Figure 1
Global circadian regulation of transcriptional activities in cyanobacteria. **(a-d)** Representative traces of various classes of rhythmic waveforms resulting from the promoter-trap experiment described in [4]. Promoter activity is measured as luminescence from a luciferase reporter. Modified from [4]. **(e)** Overexpression of the KaiC protein causes the activity of some promoters (clock-dominated, or high-amplitude, promoters) to be essentially abolished, while **(f)** clock-modulated, or low-amplitude, promoters are repressed to a basal level that is significant but non-rhythmic. Modified from [16].

KaiA stabilizes KaiC in its phosphorylated form, and KaiB antagonizes the effect of KaiA [8,13-15]. The ratio of phosphorylated to non-phosphorylated KaiC is correlated with the period at which the clock runs [15].

Continuous overexpression of KaiC was found to repress the *kaiBC* promoter (*kaiBC*p), suggesting negative feedback of KaiC on its own promoter in an analogous fashion to the situation for eukaryotic clock proteins [6]. The *kaiBC* promoter is not the only target of KaiC, however; the recent paper by Nakahira and coworkers [16] reports the unexpected result that KaiC overexpression represses the rhythms of all promoters in the *S. elongatus* genome. Intriguingly, this study identified two classes of response to KaiC repression. The first class, termed 'high amplitude' by Nakahira and coworkers [16], was exhibited by 5-10 % of the promoters, including *kaiBC*p; these promoters normally show a high-amplitude oscillation that is obliterated by KaiC overexpression (Figure 1e). This pattern reflects promoters whose expression is 'clock-dominated', with practically no basal activity at trough phases or during KaiC overexpression. The second response - exhibited by 90-95 % of promoters - is a 'clock-modulated' response, termed 'low amplitude' by Nakahira and coworkers [16] (Figure 1f). This is a lower amplitude oscillation, in which the rhythmic component is abolished by KaiC overexpression, but a significant non-rhythmic basal level remains. These results indicate that KaiC (probably as part of a complex) coordinates genome-wide gene expression; the majority of genes have significant basal activity and are rhythmically modulated by the KaiABC oscillator, while in a smaller subset of genes, the oscillator dominates transcriptional activity (Figure 1e,f) [16]. This latter class might turn out to contain genes that encode proteins intrinsically involved in the cell's circadian-clock system.

Considerable evidence indicates that circadian feedback loops in eukaryotes are autoregulatory, whereby clock proteins directly or indirectly regulate the activity of their own genes' promoters [17]. It was therefore a surprise to discover that the *kai* promoters are dispensable; Kai proteins can be expressed from a heterologous promoter and the cyanobacterial clock ticks along unperturbed [15,16]. The cyanobacterial transcriptional apparatus recognizes the heterologous promoter (in this case *trc*p from *E. coli*), but *trc*p is obviously not a promoter that evolved in conjunction with cyanobacterial clock genes. We first reported the functional replacement of *kaiBC*p [15], and now Nakahira and coworkers [16] report the functional replacement of both *kaiA*p and *kaiBC*p. Both studies found that expression of the Kai proteins needs to be within a permissive window of intracellular concentration to permit rhythmicity [15,16]. Thus, the circadian feedback loop in cyanobacteria does not require negative feedback of clock proteins upon specific clock promoters; apparently all that is required is the expression of an

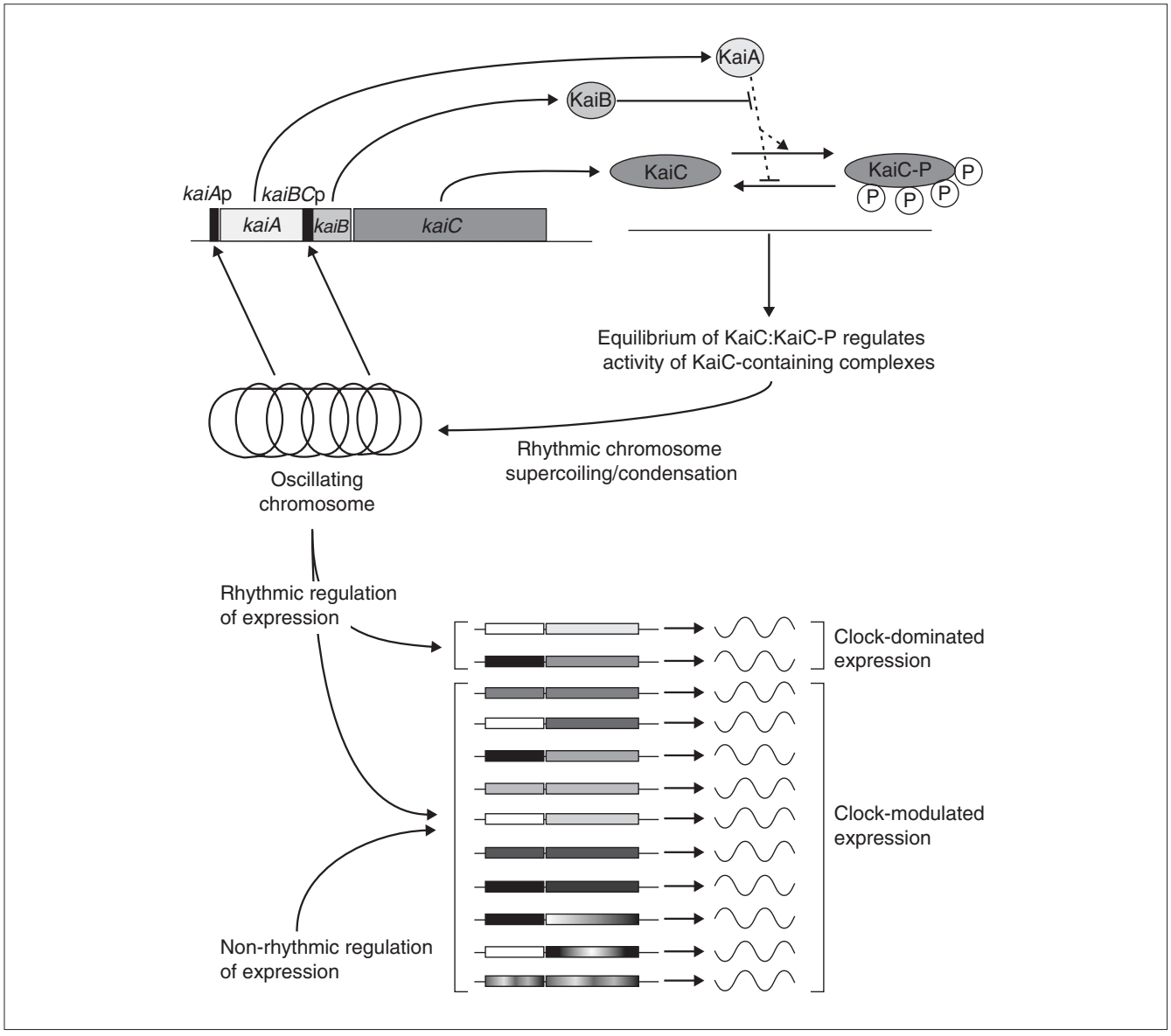

Figure 2
The 'oscilloid' model for the circadian system of cyanobacteria. KaiA, KaiB, and KaiC are synthesized from the *kaiABC* cluster using two promoters: *kaiA*p (driving expression of KaiA) and *kaiBC*p (driving expression of a dicistronic mRNA encoding KaiB and KaiC). KaiA promotes the phosphorylation of KaiC and inhibits its dephosphorylation, while KaiB antagonizes the actions of KaiA. KaiC phosphorylation is coincident with the formation of a KaiC-containing complex that mediates rhythmic and global changes in the status of the chromosome. These changes in chromosomal status influence the transcriptional activity of all promoters (including *kai* promoters) in the chromosome so that there are global circadian changes in gene expression. Approximately 10% of promoters in the organism receive only the rhythmic input and are clock-dominated, or high-amplitude (including *kaiBC*p), and the remaining 90% of promoters (clock-modulated, or low-amplitude; including *kaiA*p) receive both rhythmic input and basal non-oscillatory input. Modified from [15,16,22].

appropriate level of Kai proteins. Even temperature compensation - a defining characteristic of circadian clocks - is preserved when *trc*p replaces *kaiBC*p [16].

An oscilloid model for the circadian system

The pervasiveness of rhythmic transcriptional activity, and the fact that the clockwork does not require specific clock-gene promoters, suggests a broadly global mechanism for the cyanobacterial clock system. But what is the basis of this global regulation? One possibility could be rhythmic control by RNA polymerase sigma subunits, which often determine the promoter specificity of the polymerase. But studies of sigma subunits in cyanobacteria have not yielded explanations for global regulation [18]. An alternative is the possibility that chromosomal topology is involved. The chromosome in most bacteria is

organized into a 'nucleoid', which has a highly organized architecture based on condensation and coiling of DNA [19]. It is well known that changes in the local supercoiling status of DNA can affect the transcriptional rate of genes [20], and our findings concerning the behavior of promoters in cyanobacteria support those observations [21]. We proposed in 2001 that KaiC might mediate both its own negative feedback regulation and global regulation of the cyanobacterial genome by orchestrating oscillations in the condensation and/or supercoiling status of the entire cyanobacterial chromosome [22].

The most recent findings from our lab [15] and the lab of Susan Golden [21], in addition to the study of Nakahira and coworkers [16], are consistent with this hypothesis, namely that the condensation or supercoiling status of the cyanobacterial chromosome rhythmically changes such that it becomes an oscillating nucleoid, or 'oscilloid' (Figure 2). There is already a precedent for daily rhythms of topology in the chloroplast chromosome of the eukaryotic alga *Chlamydomonas* [23]. In cyanobacteria, we postulated that these topological oscillations promote rhythmic modulation of the transcription rates of all genes, accounting for the global regulation of gene expression [22]. Gene-specific *cis*-regulatory elements that mediate rhythmic gene expression might therefore be (at least partially) responsive to chromosomal status rather than exclusively to *trans* factors, leading to clock-dominated and clock-modulated expression patterns (Figures 1 and 2). In addition, heterologous promoters (for example *E. coli trc*p) that are integrated into the chromosome are driven rhythmically because they are also subjected to the oscillating chromosomal status [15,16]. Finally, KaiC (or, most likely, a KaiC-containing protein complex) is a key player in regulating these changes of chromosomal status [15,16], and the phosphorylation status of KaiC is important in the regulation of this complex's activity (Figure 2) [8,15].

At present, it appears that the clock system in cyanobacteria is different from that in eukaryotes, and that changes in chromosomal topology could be a key element. In the fullness of time, however, we might find rhythmic modulation of chromosomal structure to be important in eukaryotic clock regulation - indeed, suggestive evidence for that hypothesis already exists for the mammalian clock [24]. If this proves to be the case, the investigations of the cyanobacterial clock may lead to fundamental insights that are broadly applicable to all organisms.

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