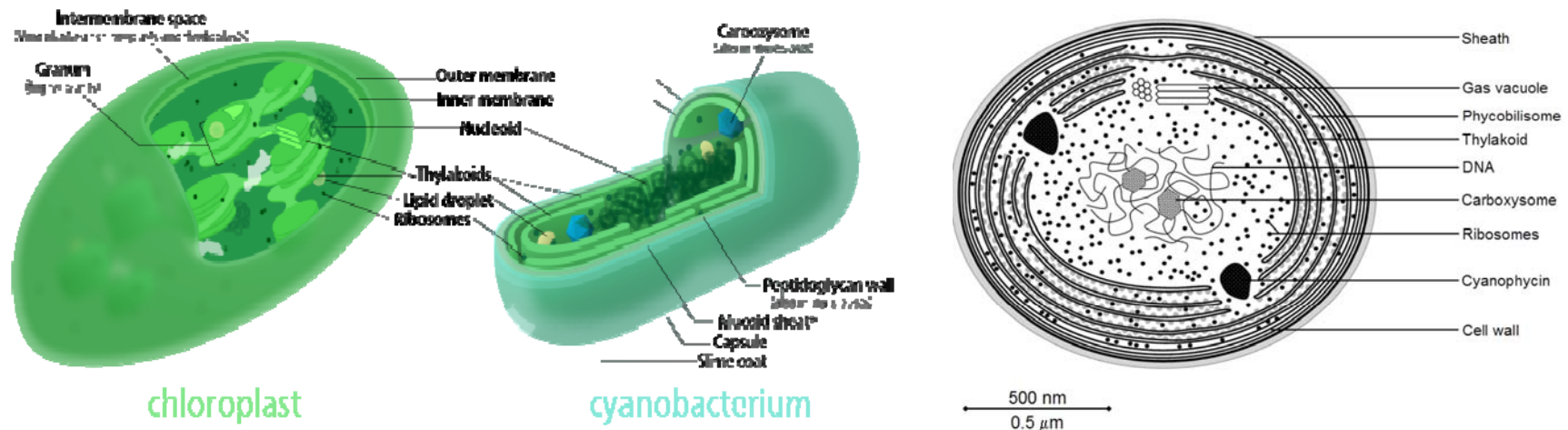


Circadian Rhythm in Cyanobacteria (*Synechococcus elongatus*)



- Single celled prokaryotic organism
- Photosynthesis is its only means of growth
- Small genome (approximately 2800 genes)
- Take up foreign DNA naturally
- Easy to knock genes out, increase expression of genes
- Robust circadian rhythms of gene expression

Working definition of Circadian System

- Approximately 24 hour cycle
- Entrainment by external signal
 - Light and dark
 - Warmer and colder
- Maintained in the absence of external signal (free run)
- Temperature compensated

Function of Clocks

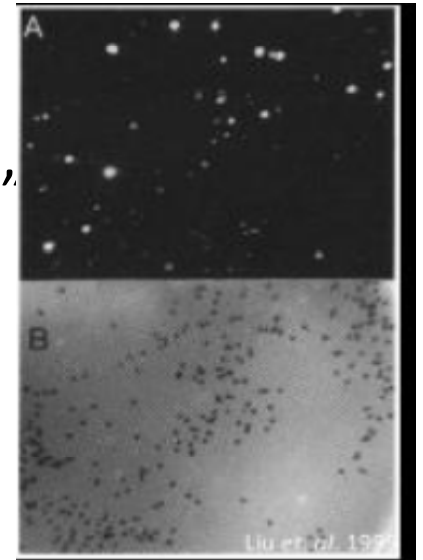
- control of organism's
 - Gene expression
 - Metabolism
 - Activity cycles
- Optimize activity to environment
- Out of synchronize clock = less fitness

Cyanobacterial Circadian Rhythms

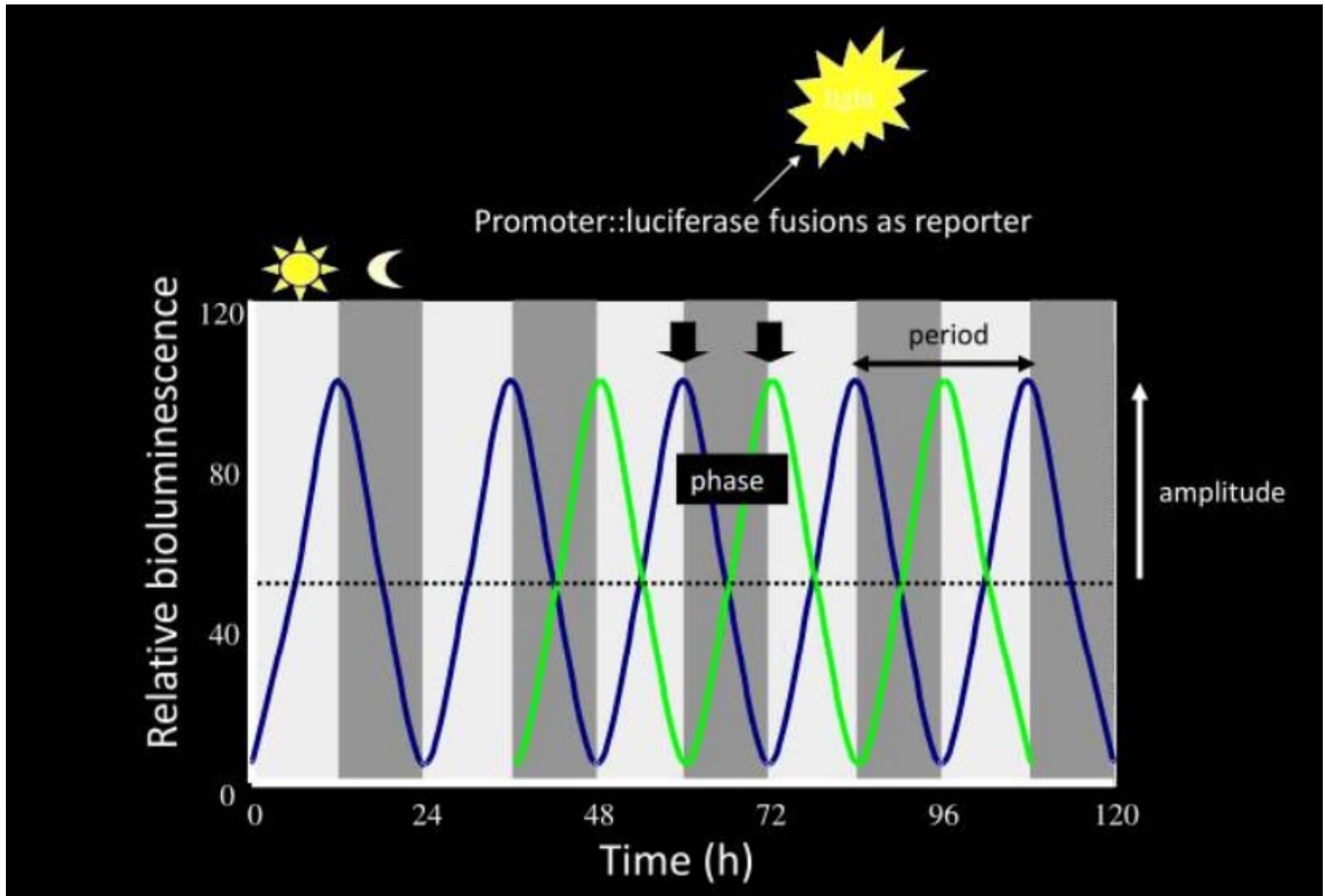
- Nitrogen fixation
- Photosynthesis
- Amino acid uptake
- Gene expression
- Timing of cytokinesis
- Confers fitness to progeny

How Many Genes Are Under Circadian Control ?

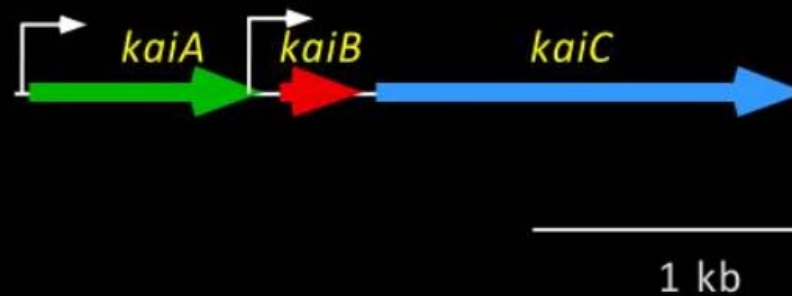
- 10 percent detectable luminescence (>20,000 colonies screened, closer to 30,000 ?)
- Colonies separated into “Bright” (≥ 130 CPM) and “dims” (≤ 130 CPM)
- Bioluminescence rhythm detected in
 - 798 selected Bright (almost every bright colony)
 - 40 selected Dims
- Control:
 - Southern blot indicates
 - Northern blot of known genes
 - Known rhythmic promoters also had bioluminescent rhythms
- Conclusion:
 - All genes of *S. elongatus* are rhythmic
- Assumptions:
 - The non-trackable clones are rhythmic
 - Insertions are really random, and low chance of duplication



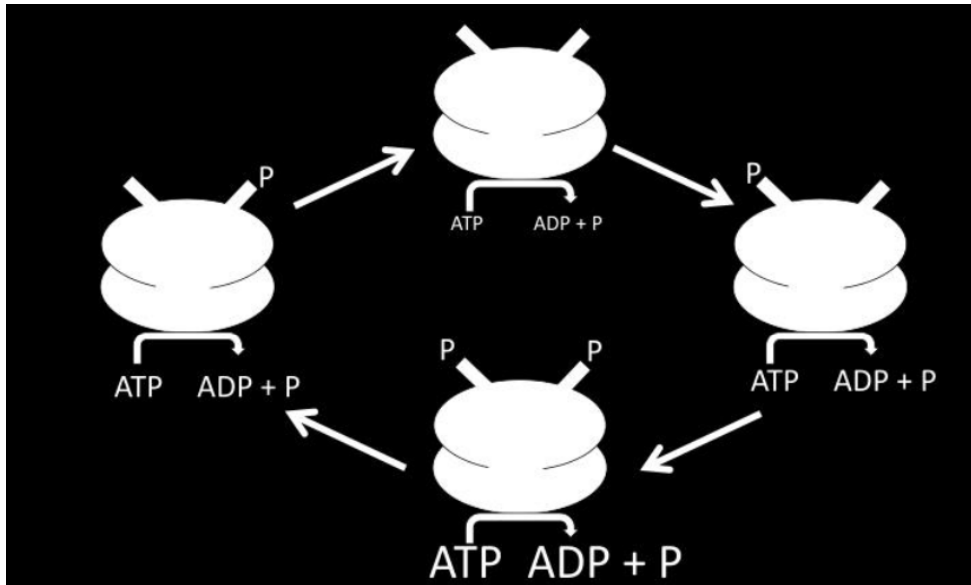
Circadian rhythm of gene expression



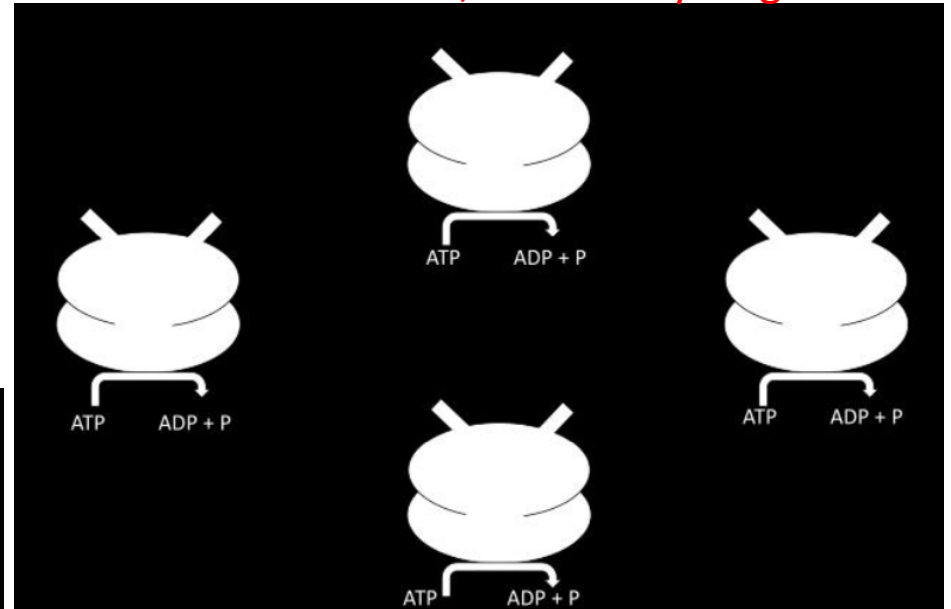
kaiA, *kaiBC* Required for Functional Clock



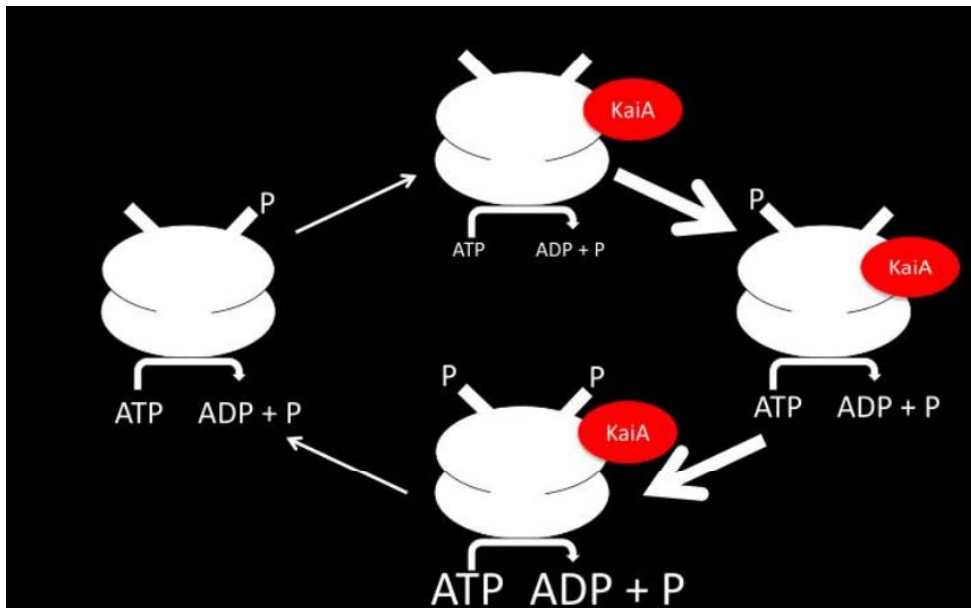
KaiC is Autophosphatase, Autokine, ATPase

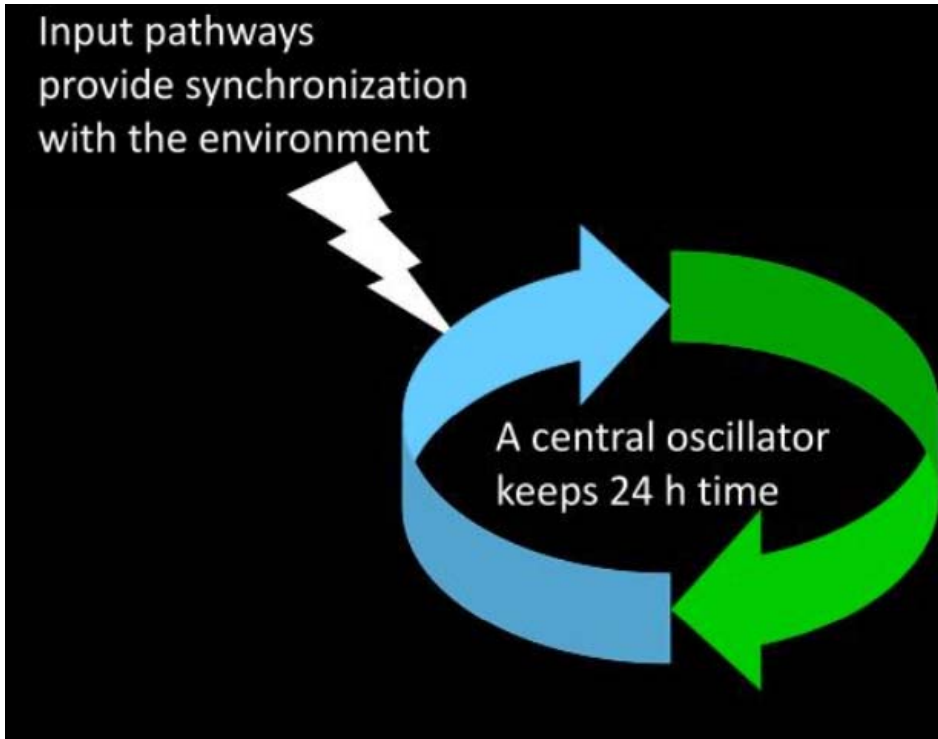


but with out Kai A & B, KaiC no cycling occurs

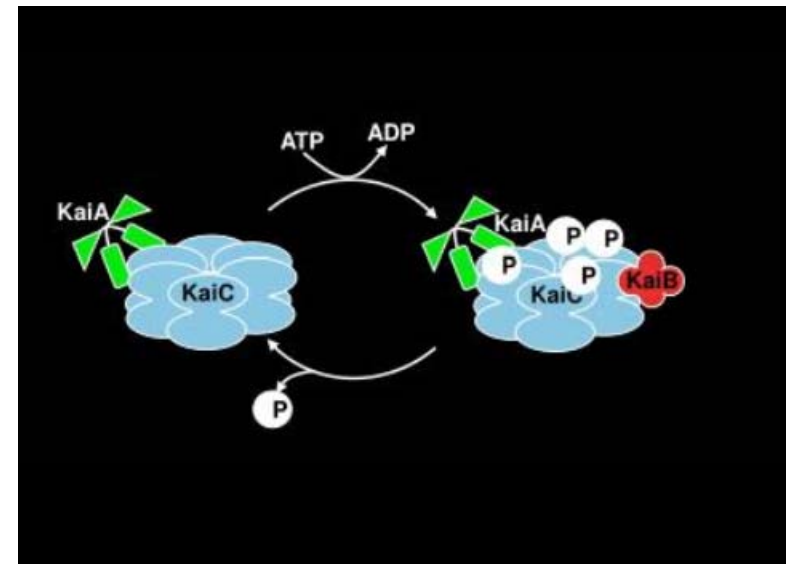


KaiA stimulates phosphorylation, ATPase activity



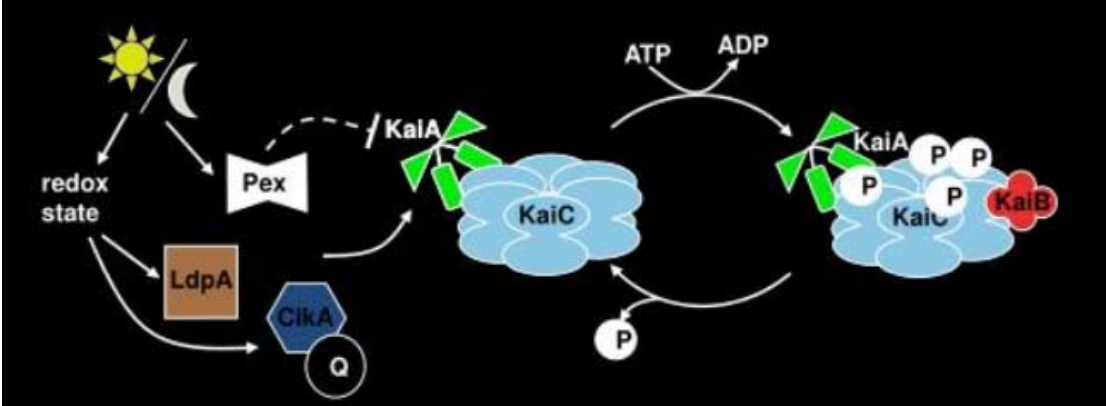


The central oscillator is not the clock

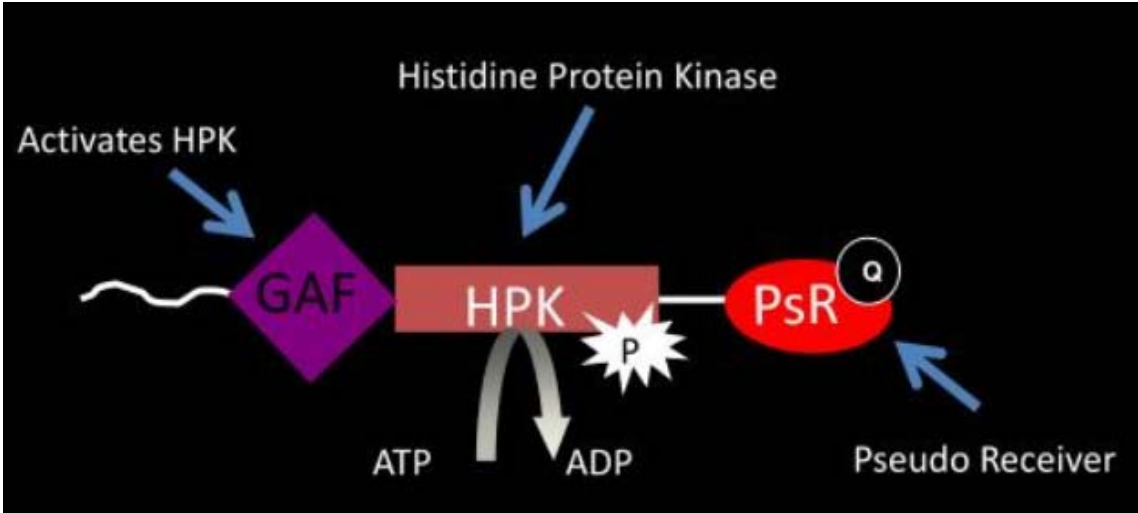


<https://www.slideserve.com/blaine/circadian-rhythms-in-synechococcus-elongatus>

Expansion out from the Central Oscillator

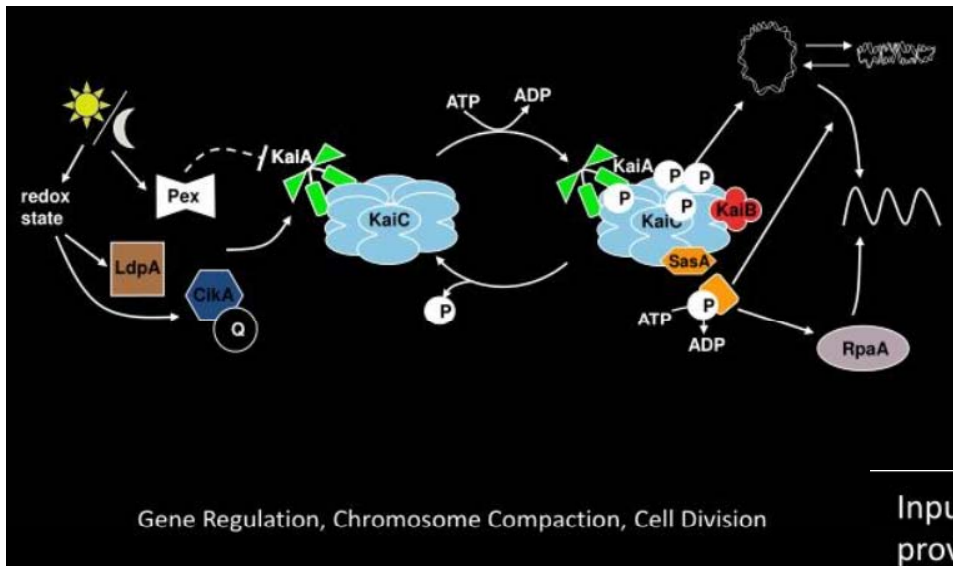


Feature of CikA

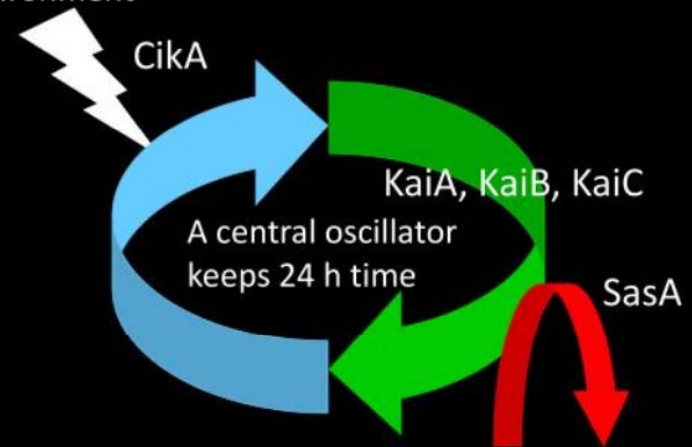


<https://www.slideserve.com/blaine/circadian-rhythms-in-synechococcus-elongatus>

Clock output

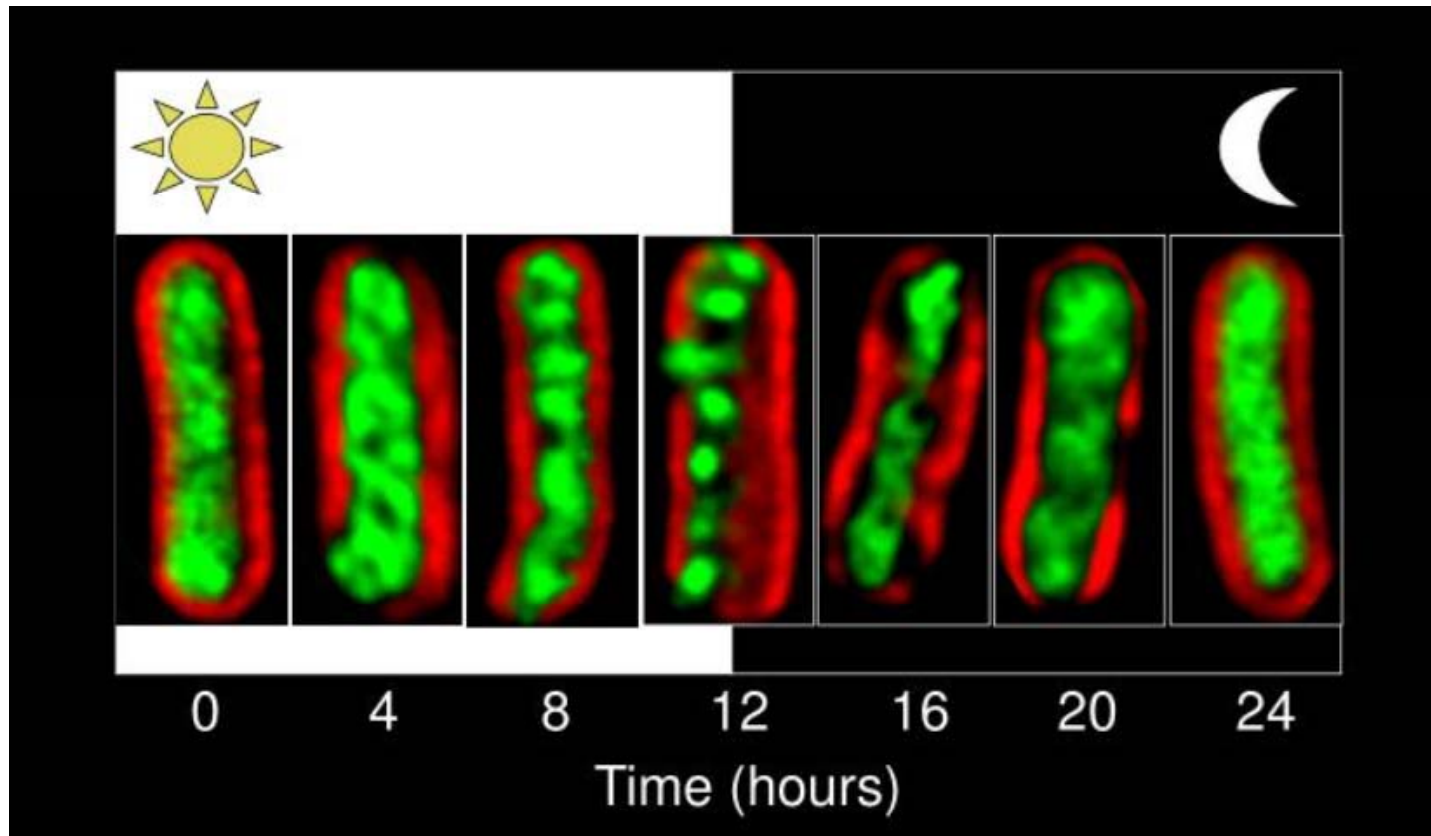


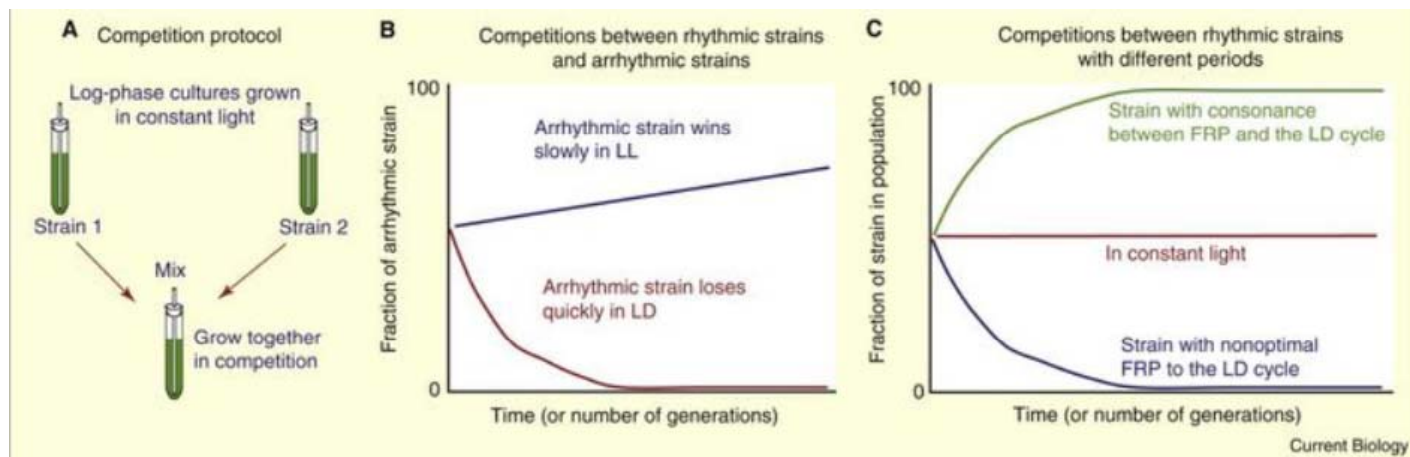
Input pathways
provide synchronization
with the environment



Output pathways
transmit temporal information
to clock-controlled behaviors

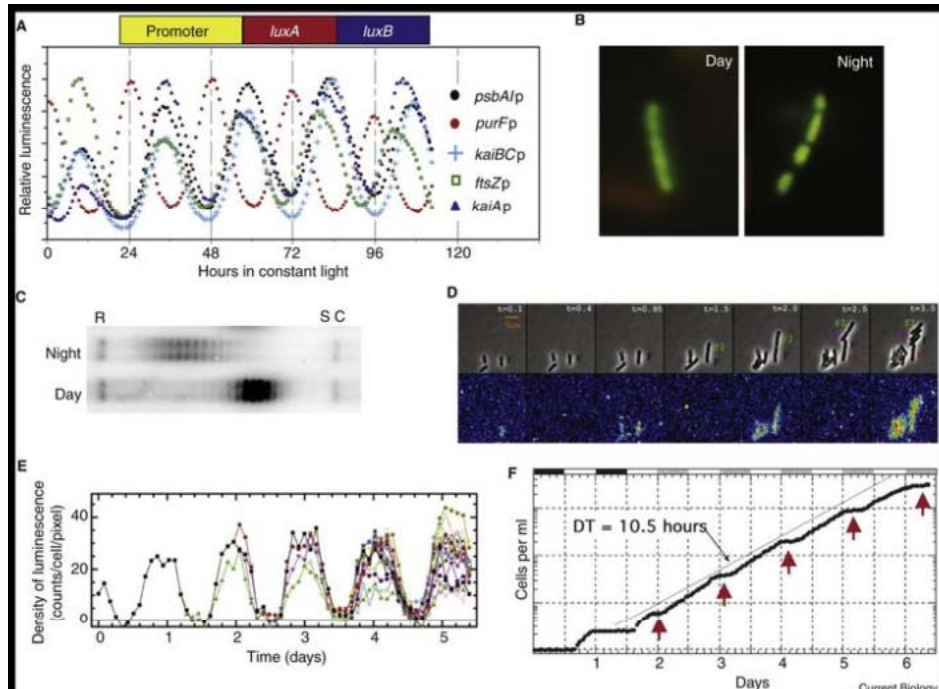
Chromosome compaction in *S. elongatus*





Competition between circadian strains in different LD cycles.

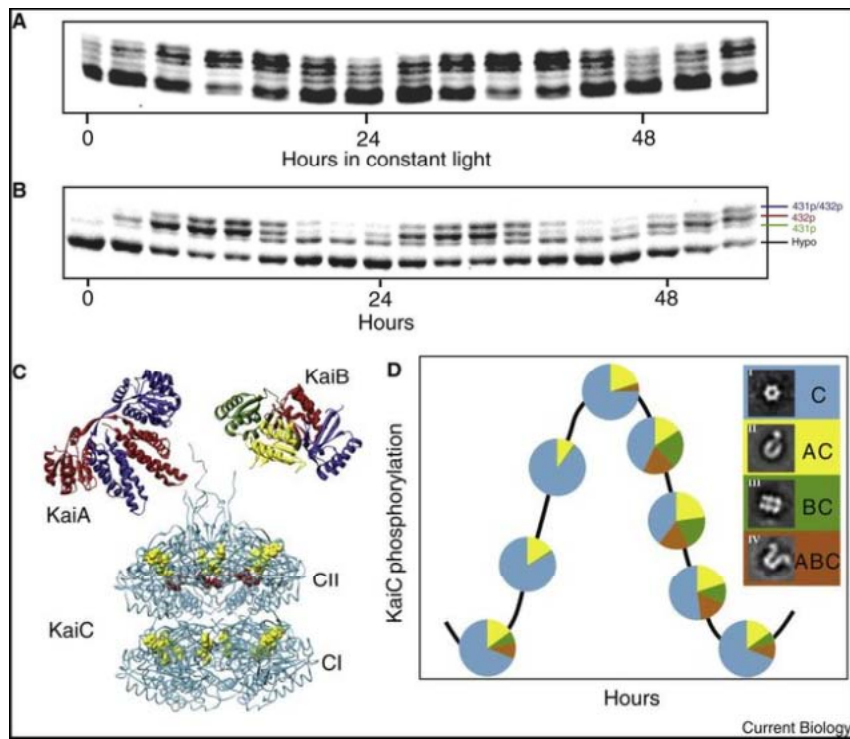
- A. Different strains of *S. elongatus* were mixed together in batch cultures and grown in competition under different LD cycles. Every 8 days, the cultures were diluted with fresh medium. At various times during the competition, aliquots were plated as single colonies and their luminescence rhythms were monitored to determine the frequency distribution of the different circadian phenotypes.
- B. In competitions between wild-type and arrhythmic strains, the arrhythmic strain is rapidly out-competed in LD cycles, but slowly defeats wild-type strains in constant light (LL, nonselective conditions).
- C. In competitions among strains that are rhythmic, the strain whose endogenous free-running period (FRP) most closely matches that of the environmental LD cycle is able to out-compete strains with a non-optimal FRP. In LL, all the strains were able to maintain their initial fraction in the population.



Circadian rhythms in *S. elongatus*.

(A) Circadian rhythms of gene expression monitored as rhythms of luminescence emanating from cells transformed with bacterial luciferase (*luxA*/*luxB*) fused to the promoters for the *psbA*, *kaiA*, *kaiBC*, *purF*, and *ftsZ* genes. (B) Circadian rhythm of chromosome compaction as visualized by a fluorescent DNA-binding dye. The chromosome is more compacted in the subjective night than in the subjective day; *S. elongatus* has several copies of the same chromosome [28] that appear as fluorescent balls when compacted as seen in the night-phase cells in this panel. (C) Chromosome topology shows a circadian rhythm as assayed by supercoiling of an endogenous plasmid. Topoisomers of the plasmid are more relaxed (R) in the subjective night and are more supercoiled (SC) in the subjective day. (D,E) Circadian rhythms of luminescence in single cyanobacterial cells. (D) Micrographs of cyanobacterial cells at different times in constant light. The upper panels are bright field images showing growth and cell division as a function of approximate circadian time and the lower panels show the luminescence emanating from these cells (the luminescence reporter is the *psbA* promoter driving expression of bacterial luciferase). (E) Quantification of bioluminescence from a single cell as it divides into multiple cells as a function of time in constant light; starting at day 1.5, there are two differently colored traces as a result of cell division, the next division occurs at day 2.0, and so on (panels D and E have been reproduced with permission from). (F) Cell division of a population of *S. elongatus* cells. The first 36 hours are in a light–dark cycle (phases of darkness are indicated by the black bars at the top of the panel). Thereafter, the cells are in constant light. Cell number is plotted on the y-axis and the phases in constant light when the cells stop dividing are indicated by the red arrowheads. The average doubling time (DT) of this culture was 10.5 hours, as indicated by the diagonal line.

Ref: Johnson et al. 2008. A cyanobacterial circadian clockwork. Current Biology. 18(17):R816-R825.



Rhythms of KaiC proteins in vivo and in vitro.

(A) Rhythmic KaiC phosphorylation in vivo at different times in constant light. Samples were collected every 4 hours in constant light and processed for SDS-PAGE and immunoblotting. The lowest band is hypo-phosphorylated KaiC and the upper bands are various forms of phosphorylated KaiC. Hyper-phosphorylated KaiC is most prevalent at hours 8–16 and 32–40. (B) Rhythmic KaiC phosphorylation in the in vitro reaction. Purified KaiA, KaiB, and KaiC are combined with ATP in vitro and samples collected every 3 hours and processed for SDS-PAGE and staining. Four bands are obvious in these samples: hypo-phosphorylated KaiC (Hypo) and KaiC phosphorylated at the S431 (431p), T432 (432p), or S431/T432 (431p/432p) residues. (C) 3D structures of the KaiA dimer (monomer MW = 30 kDa; PDB: 1R8J), the KaiB tetramer (monomer MW = 11 kDa; PDB: 1WWJ), and the KaiC hexamer (monomer MW = 58 kDa; PDB: 2GBL). In the cases of KaiA and KaiB, various subunits are distinguished by different colors. In the case of KaiC, all the subunits are shown in the same color, but the bound ATP molecules are shown in yellow and the S431/T432 phosphorylation sites are shown in red. Each monomer of KaiC has a CI and CII domain, leading to a CI ‘end’ and a CII ‘end’ of KaiC. Available evidence indicates that KaiA and KaiB interact with KaiC at the CII end. In the case of KaiA, this interaction is probably via the carboxy-terminal peptides shown as tentacles extending upwards from the CII end. (D) Rhythms of KaiA/B/C complexes during the in vitro reaction as visualized by electron microscopy of the protein mixtures. The complexes themselves are shown in the boxes to the right, with color coding that corresponds with the pie charts throughout the KaiC phosphorylation rhythm. For example, only free KaiC and KaiA–KaiC complexes are present during the rising phase of KaiC phosphorylation, while free KaiC, KaiA–KaiC, KaiB–KaiC, and KaiA–KaiB–KaiC are all present during the dephosphorylation phase (modified from

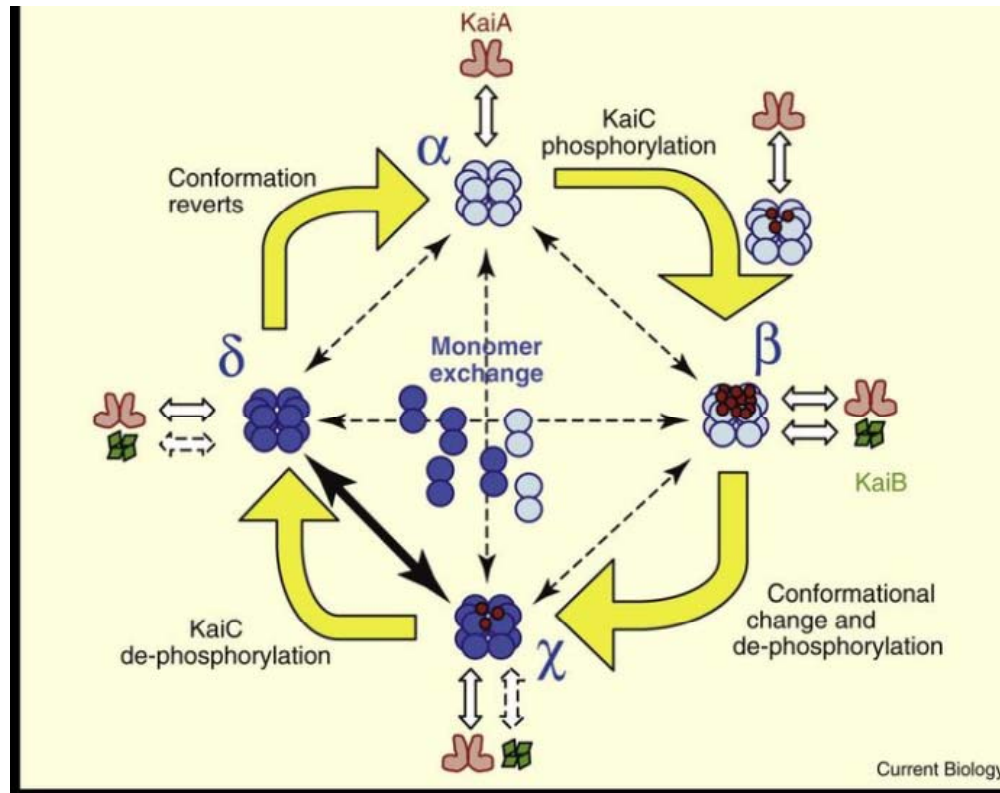
A model for the phosphorylation cycle of a KaiC hexamer and its association with KaiA and KaiB.

A KaiC monomer is shown as a double circle that can form a hexamer.

KaiC hexamers can associate or dissociate with KaiA and/or KaiB.

KaiC hexamers are depicted in two conformational states: a default KaiC status (light blue color) and an altered KaiC* state that has undergone a conformational change (darker blue color).

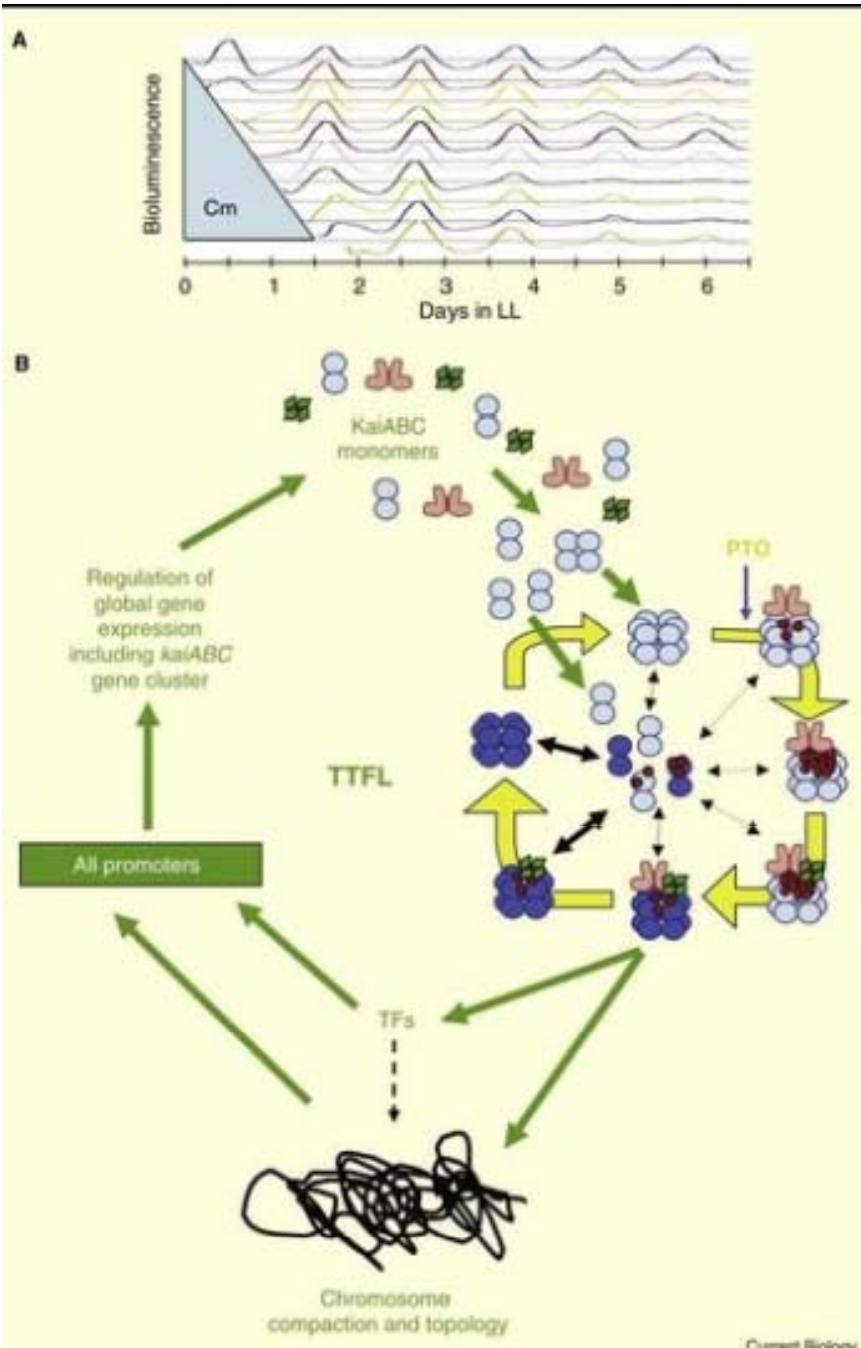
Red dots are phosphates attached to the S431 and T432 phosphorylation sites on KaiC.



A model for the phosphorylation cycle of a KaiC hexamer and its association with KaiA and KaiB

Starting from a hypo-phosphorylated state (a), rapid binding and unbinding of KaiA facilitates autophosphorylation until the KaiC hexamers are hyper-phosphorylated (state b). In this model, KaiB is assumed to preferentially associate with hyper-phosphorylated KaiC; there is a simultaneous conformational change of KaiC to a new state (KaiC* depicted as a darker blue hexamer). The KaiC* hexamer dephosphorylates through states c and d until it is no longer phosphorylated, at which time it reverts to the original state (a). Robustness is maintained by synchronization of KaiC hexameric status via monomer exchange, depicted by 'dumbbell' KaiC monomers exchanging with KaiC hexamers in the central region of the figure. The rate of this exchange is highest between hexamers during the dephosphorylation phase, as indicated by the thicker exchange arrow in this phase.

Ref: Johnson et al. 2008. A Current Biology.



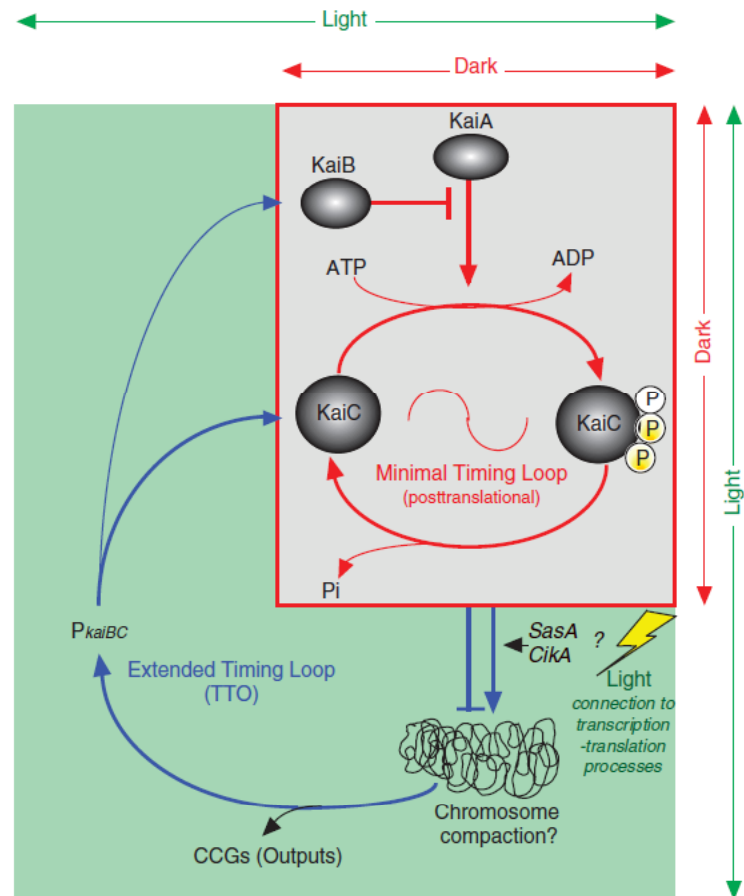
A self-sustained post-translational oscillator (PTO) embedded within a transcription and translation feedback loop.

A. Inhibiting protein synthesis does not perturb the phase of the clock. The translational inhibitor chloramphenicol (Cm) was applied to cyanobacterial cultures for various durations (blue triangle), then removed. If the clock were stopped, or otherwise perturbed by the Cm treatment, the phase of the subsequent rhythms would be expected to follow the diagonal line of the Cm wash-out. In contrast, we found that the phase of the subsequent rhythms align vertically, indicating that the cyanobacterial clock's phase was not affected by inhibition of protein synthesis.

B. A self-sustained PTO embedded within a transcription and translation feedback loop (TTFL, represented by green arrows). The PTO is shown in greater detail in Figure 4, and is represented here by yellow arrows. New synthesis of KaiC monomers feeds into the KaiABC oscillator as non-phosphorylated hexamers or as monomers that exchange into pre-existing hexamers. If the new synthesis of KaiC occurs at a phase when hexamers are predominantly hypo-phosphorylated, the oscillation of KaiC phosphorylation is reinforced (enhanced amplitude). If on the other hand, new synthesis of unphosphorylated KaiC happens at a phase when hexamers are predominantly hyper-phosphorylated, this could lead to an overall decrease in the KaiC phosphorylation status, thereby altering the phase of the KaiABC oscillator (phase shift) and/or reducing its amplitude. Some configuration of KaiC (here shown as the dephosphorylating phase, but it could be any phase of the PTO) mediates rhythmic DNA torsion/compaction and transcription factor (TF) activity to control global transcription of all promoters, including those driving expression of the essential clock genes *kaiA*, *kaiB*, and *kaiC*.

Overview of the Circadian system in Cyanobacteria

- Kai A, KaiB and KaiC interact to produce oscillation in the phosphorylation state of KaiC
- Proposed model of Kai system



Summery

- **Cyanobacteria has a functional circadian clock**
- **KaiA KaiB and KaiC comprise the central oscillator**
 - **loss of any = no circadian Clock**
 - **Oscillations can be reconstituted in vitro**
- **Time measured by Redox state(dark)**
- **Output include**
 - **Gene expression rhythm**
 - **Chromosome compaction**