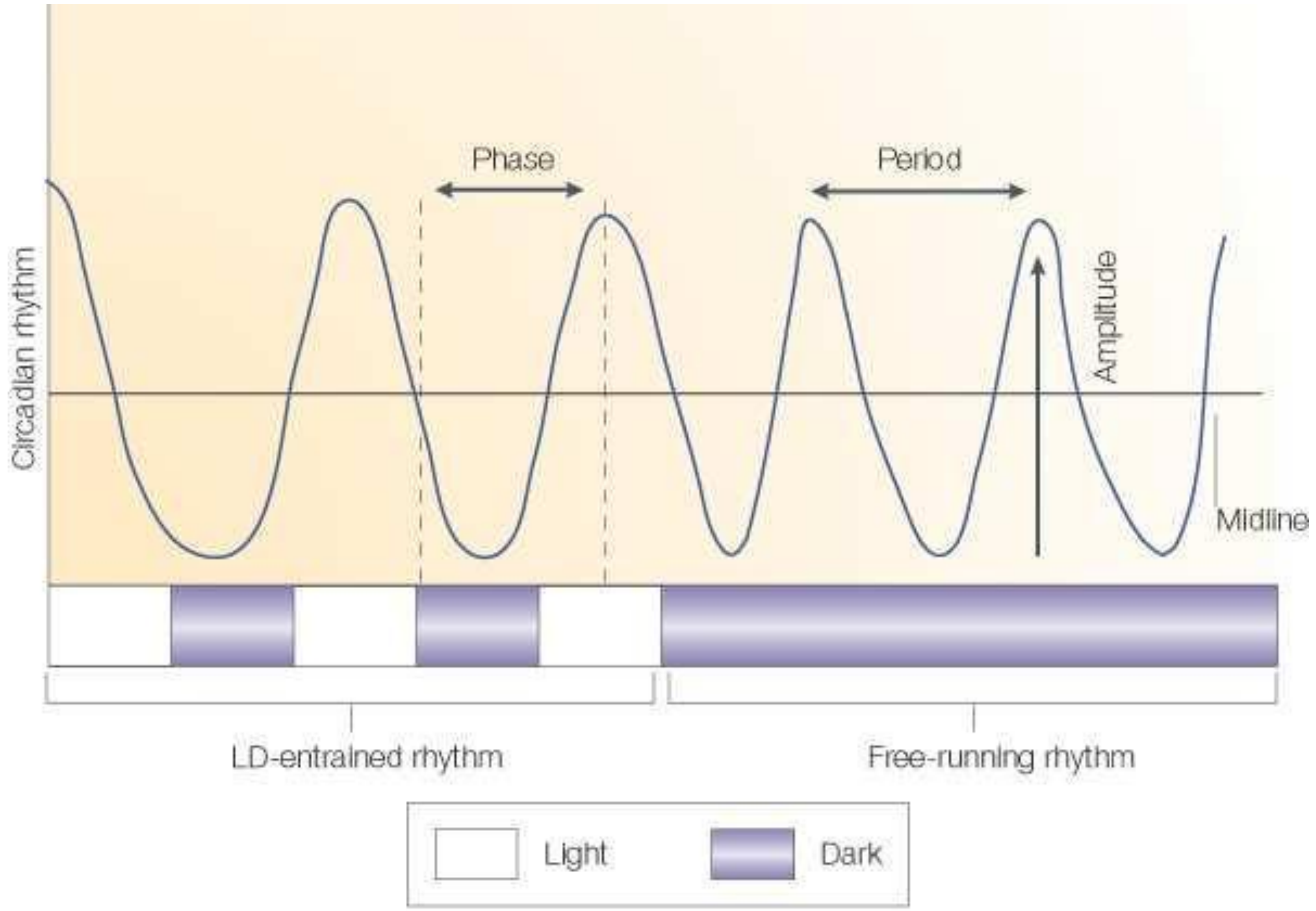
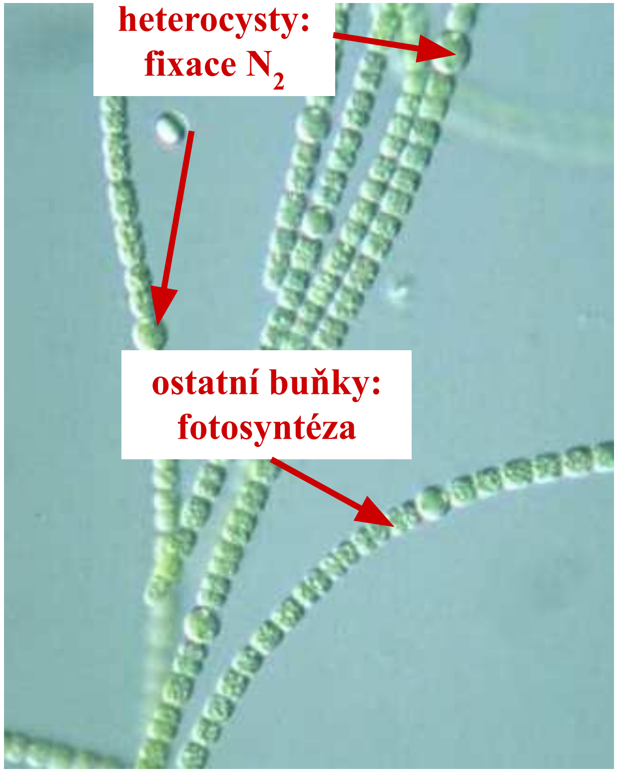
Circadian rhythm



časová kompartmentace

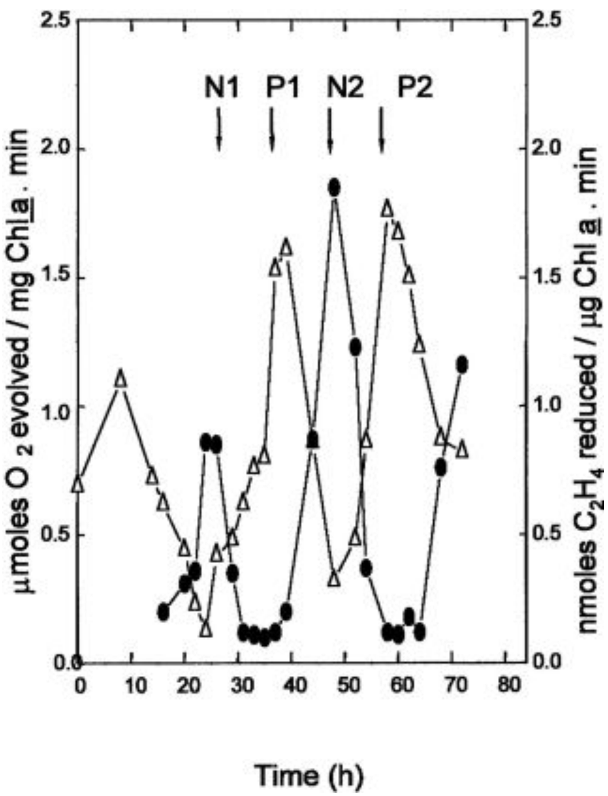
- některé druhy sinic fixují vzdušný dusík, ale nitrogenáza je silně inhibována kyslíkem
- proto je nutné **oddělit fixaci N_2 od fotosyntézy**

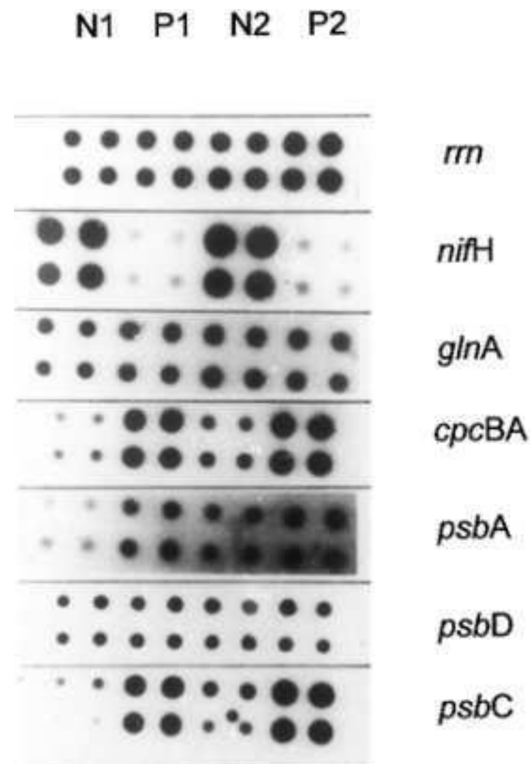


Anabaena sp.

Oscillatoria limosa

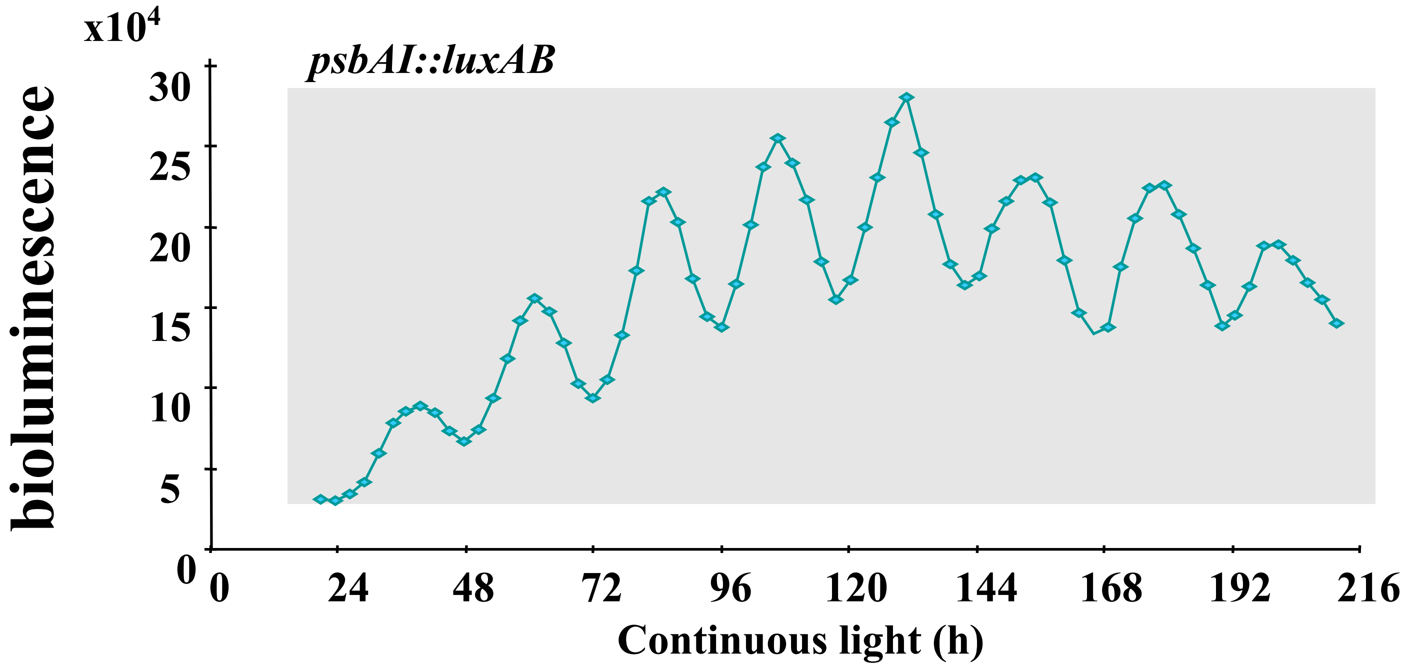
Synechococcus sp.


N1
P1
N2
P2
µmoles O 2 evolved / mg Chl a . min
nmoles C2H4 reduced / µg Chl a . min
Time (h)
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40
50
60
70
80


N1
P1
N2
P2
rrn
nifH
glnA
cpcBA
psbA
psbD
psbC

Identification of timekeeping genes

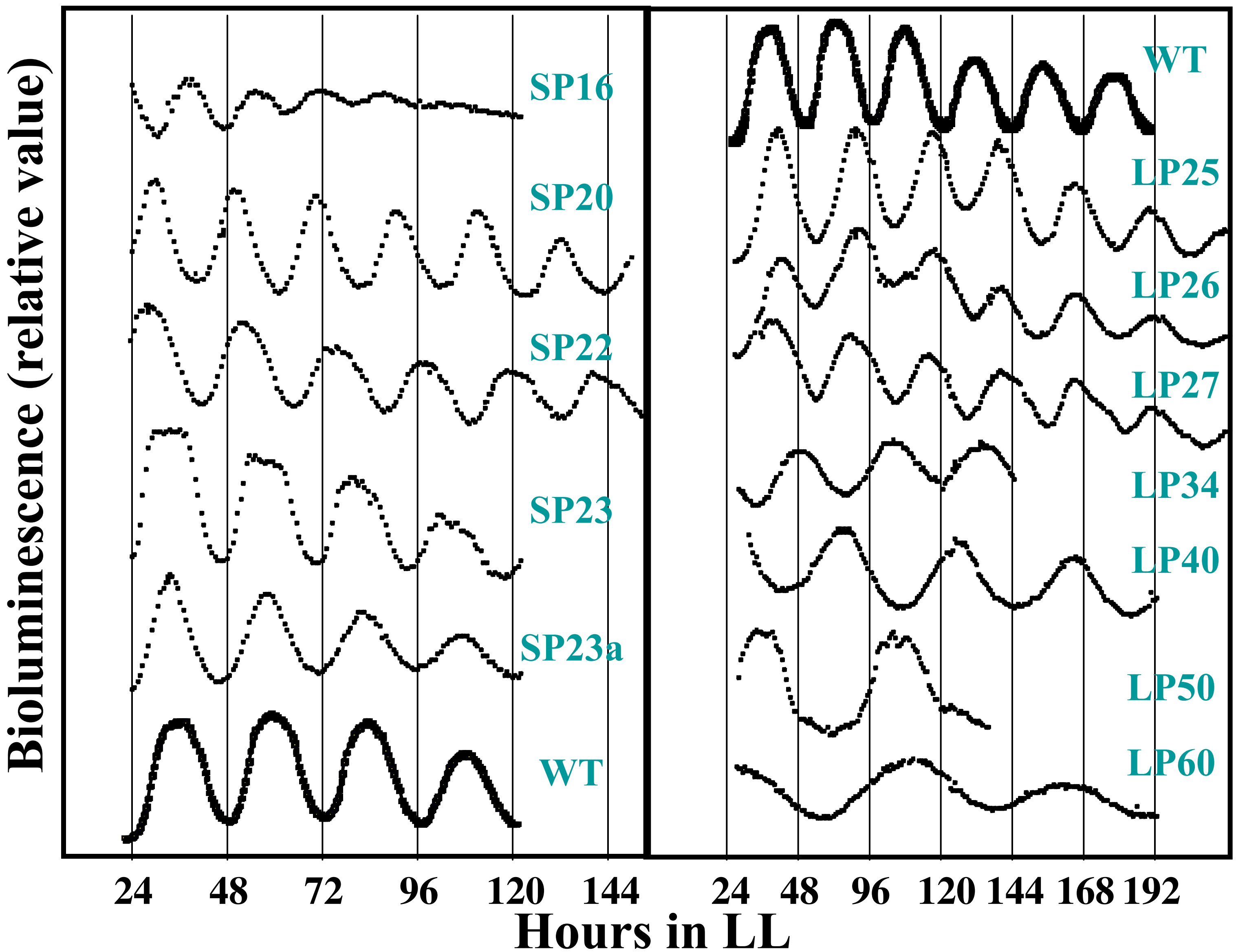
- chemical mutagenesis of a reporter strain



- screened for altered circadian gene expression

- identified arhythmic and altered period phenotypes

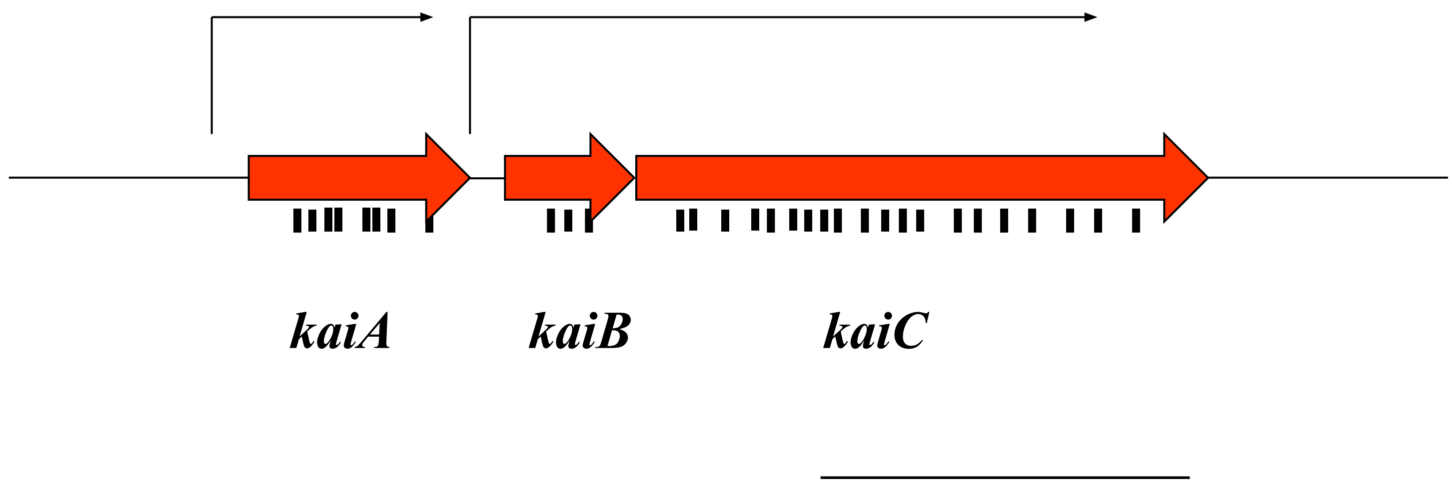
Mutant circadian rhythm phenotypes



Identification of the *kai* locus

- mutagenized a reporter strain
- screened for altered circadian expression
- identified arhythmic and altered period mutants

rescued phenotypes (3 contiguous genes)

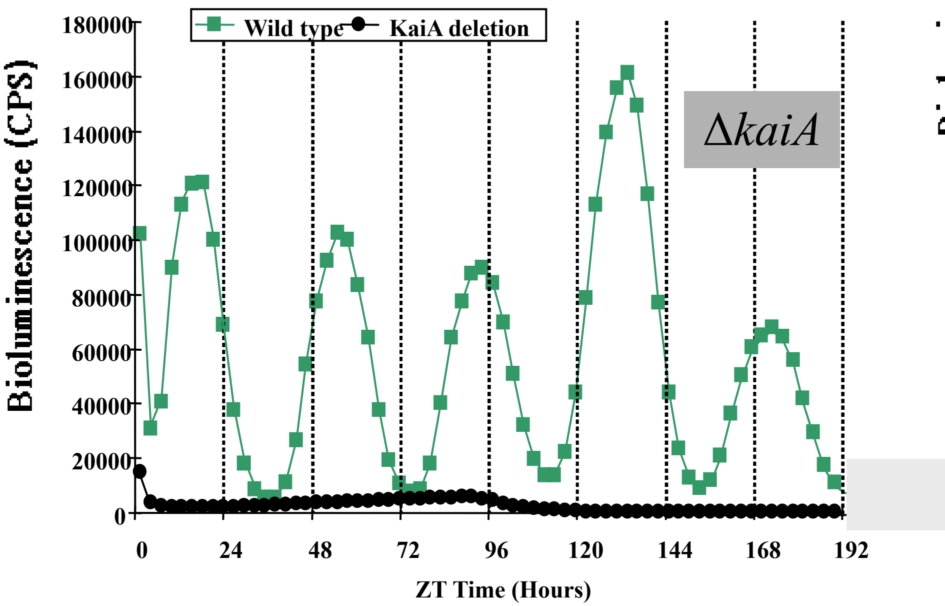


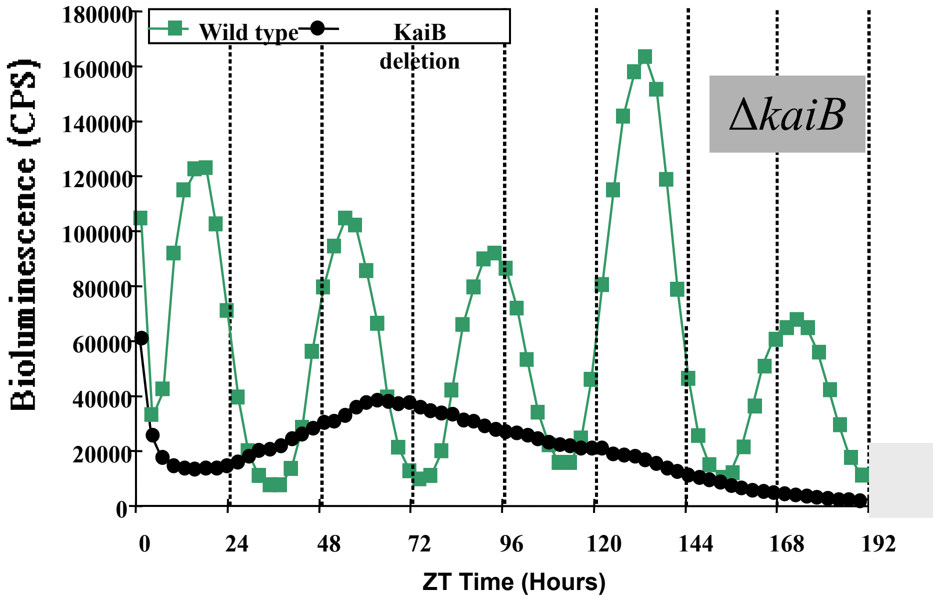
*Ishiura, M., et al. (1998) Science **281**:1519-23*

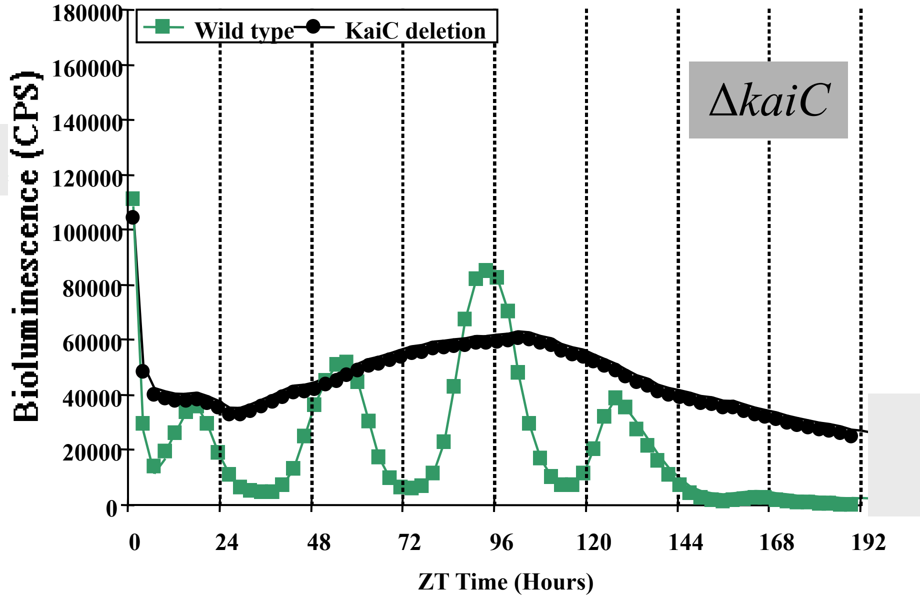
<u>Kai</u>ten means "cycle" in Japanese.

kai deletion phenotypes?

Patterns of circadian expression from *kaiB::luc*$^{+}$



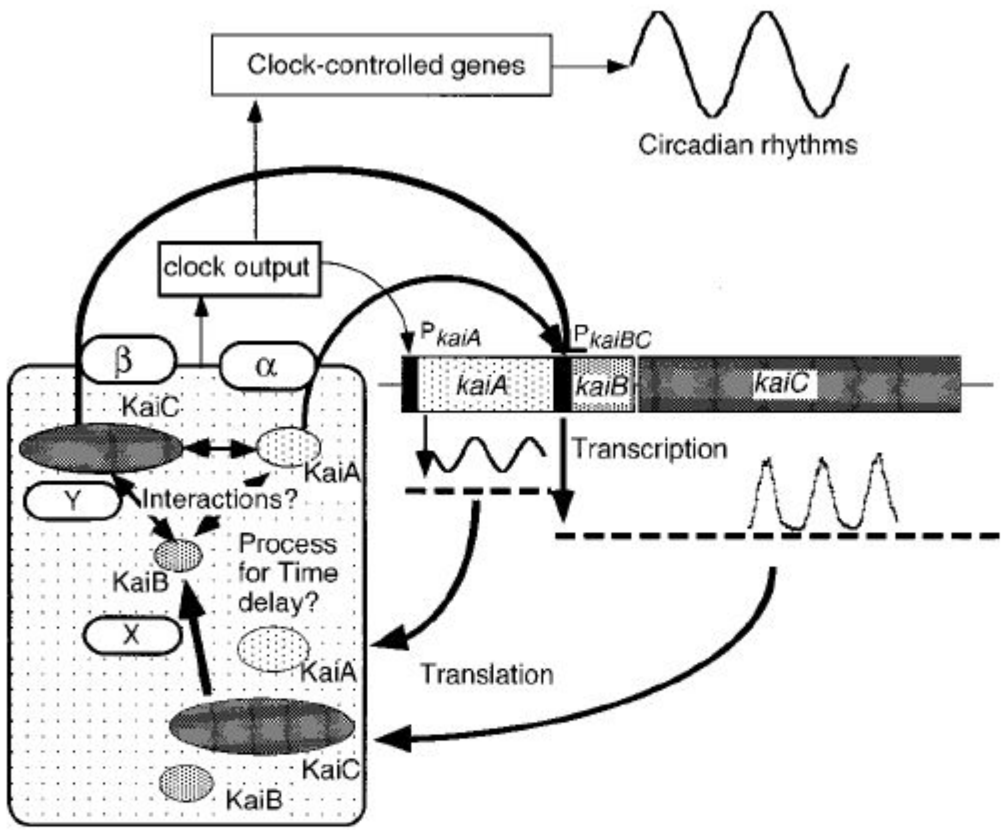




...each kai gene is essential for rhythmicity

Cirkadiánní oscilátor sinice *Synechococcus elongatus*

- KaiA, KaiB, KaiC
- první model předpokládal transkripční/translační smyčku jako u eukaryot



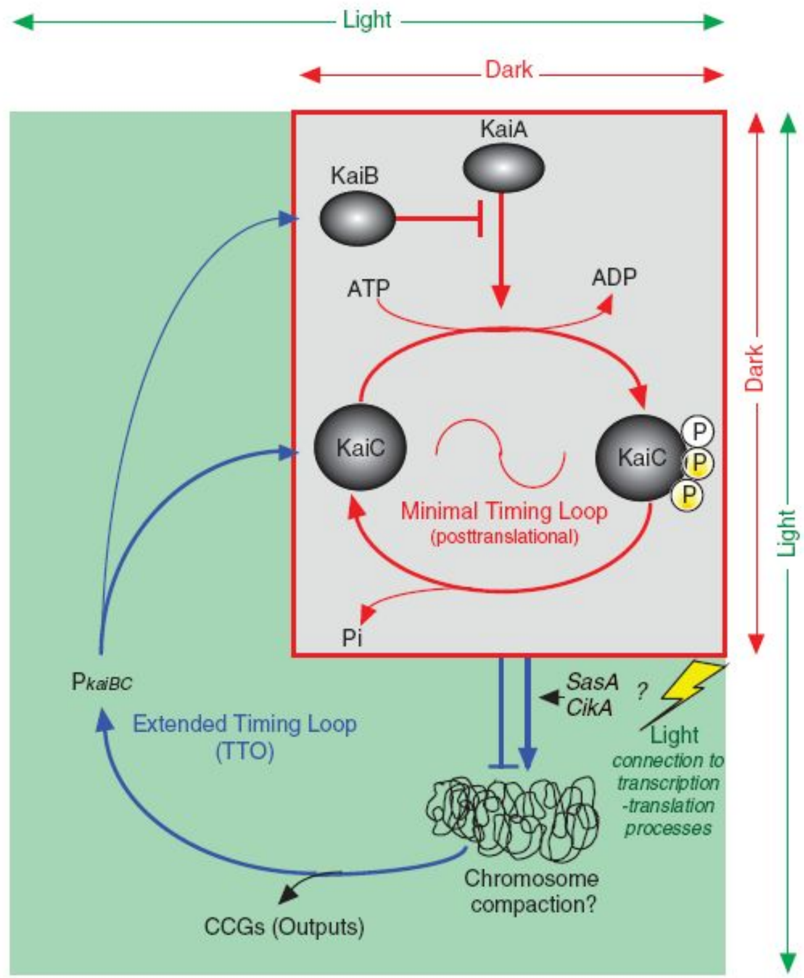
Ishiura et al.
1998

Cirkadiánní oscilátor sinice *Synechococcus elongatus*

- transkripční/translační smyčka posiluje oscilace na světle, ale oscilátor funguje (rytmicky se mění fosforylace KaiC) i ve stálé tmě a při inhibici transkripce a translace

zásadní rozdíl oproti oscilátorům eukaryot

Fig. 4. A model for the posttranslational oscillator coupled with TTO. The KaiC phosphorylation cycle can be maintained in the dark as a minimal timing loop without transcription or translation (gray area). During LL, gene expression activated by an energy supply from photosynthesis expands the oscillation to the TTO form (green area). Two histidine kinases (SasA and CikA) (*24*) might be required to connect KaiC function to a process in the general transcription mechanism, such as chromosome superhelicity (*8*), which feeds back to the *kaiBC* promoter (P_{kaiBC}) activity and regulates output gene expression globally in a circadian manner. In the dark or under nutrition-limited conditions, the posttranslational oscillator may work as a "time memory" process to ensure robust circadian organization in *Synechococcus*. Pi and CCGs indicate phosphate and clock-controlled genes, respectively.



TTO: transcription/translation oscillation

Tomita et al. 2005

Cirkadiánní oscilátor sinice *Synechococcus elongatus*

•míra fosforylace proteinu KaiC osciluje také v systému in vitro (složky: KaiC, KaiA, KaiB, ATP), a to s cirkadiánní periodou a dokonce i s teplotní kompenzací

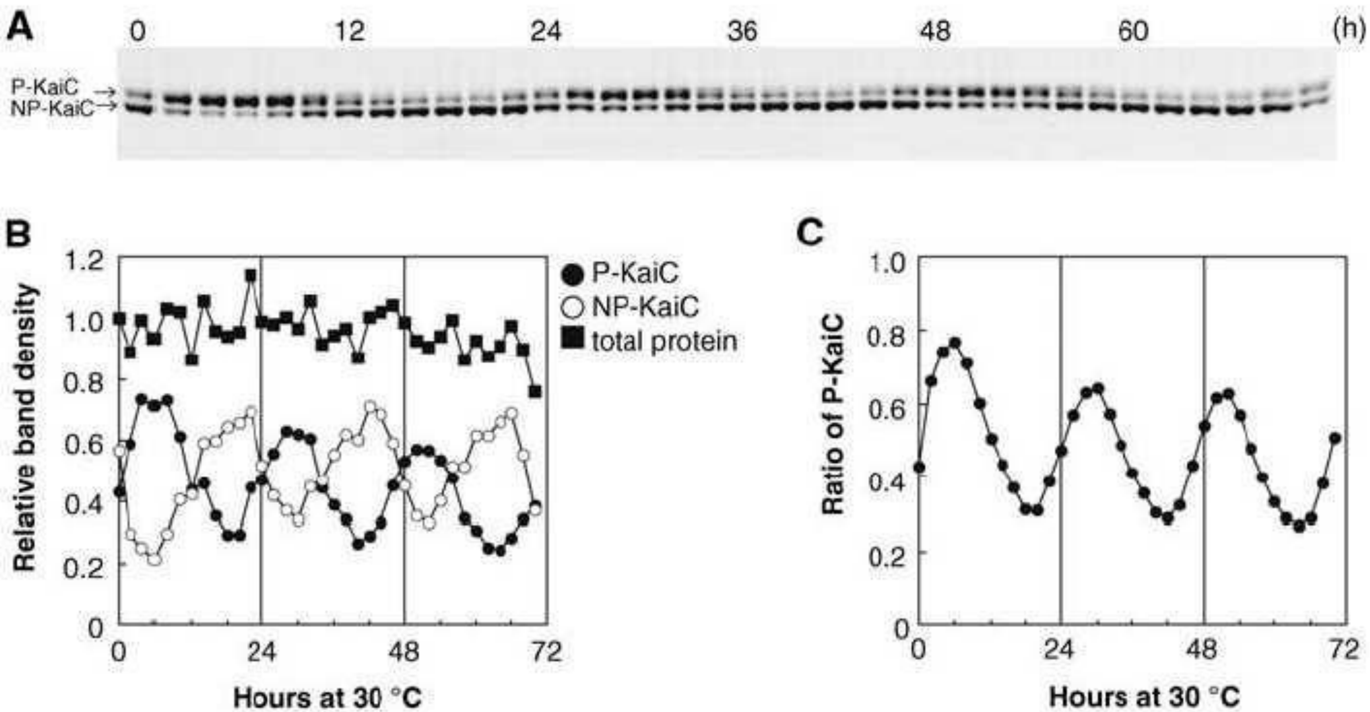
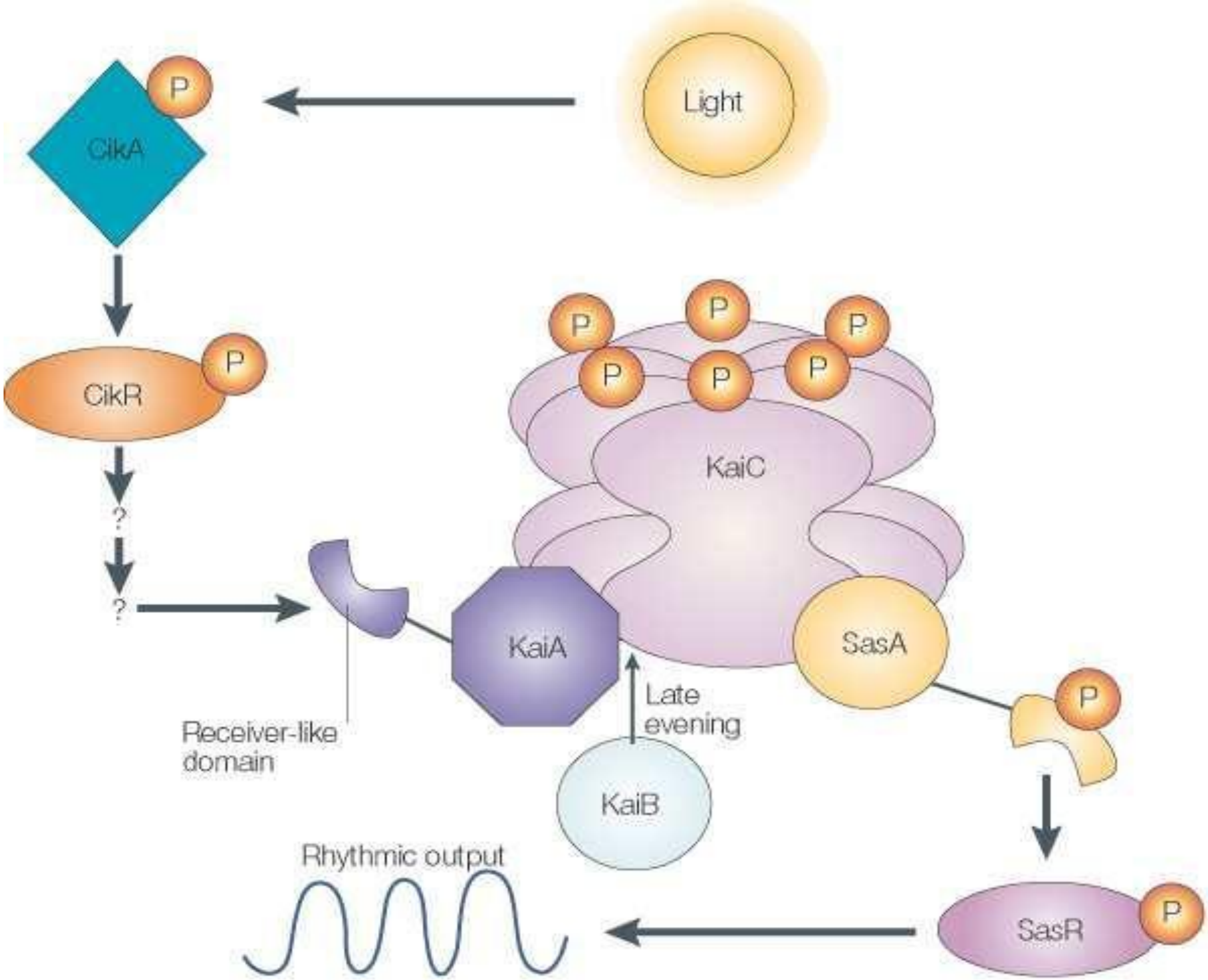


Fig. 1. In vitro oscillation of KaiC phosphorylation. (A) Recombinant KaiC proteins (0.2 μg/μl) were incubated with KaiA (0.05 μg/μl) and KaiB (0.05 μg/μl) in the presence of ATP (1 mM) (*17*). Aliquots (3 μl each) of the reaction mixtures were collected every 2 hours and subjected to SDS–polyacrylamide electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue staining. The upper and lower bands correspond to phosphorylated (P-KaiC) and unphosphorylated KaiC (NP-KaiC), respectively (*6*). (B and C) NIH image software was used to perform densitometric analysis of data (*5*) in (A). The relative densities of total, phosphorylated, and unphosphorylated KaiC are plotted in (B), and the ratios of P-KaiC to total KaiC are plotted in (C).

Nakajima et al. 2005


Light
P
CikA
CikR
?
?
KaiC
KaiA
SasA
Receiver-like
domain
Late
evening
KaiB
Rhythmic output
SasR