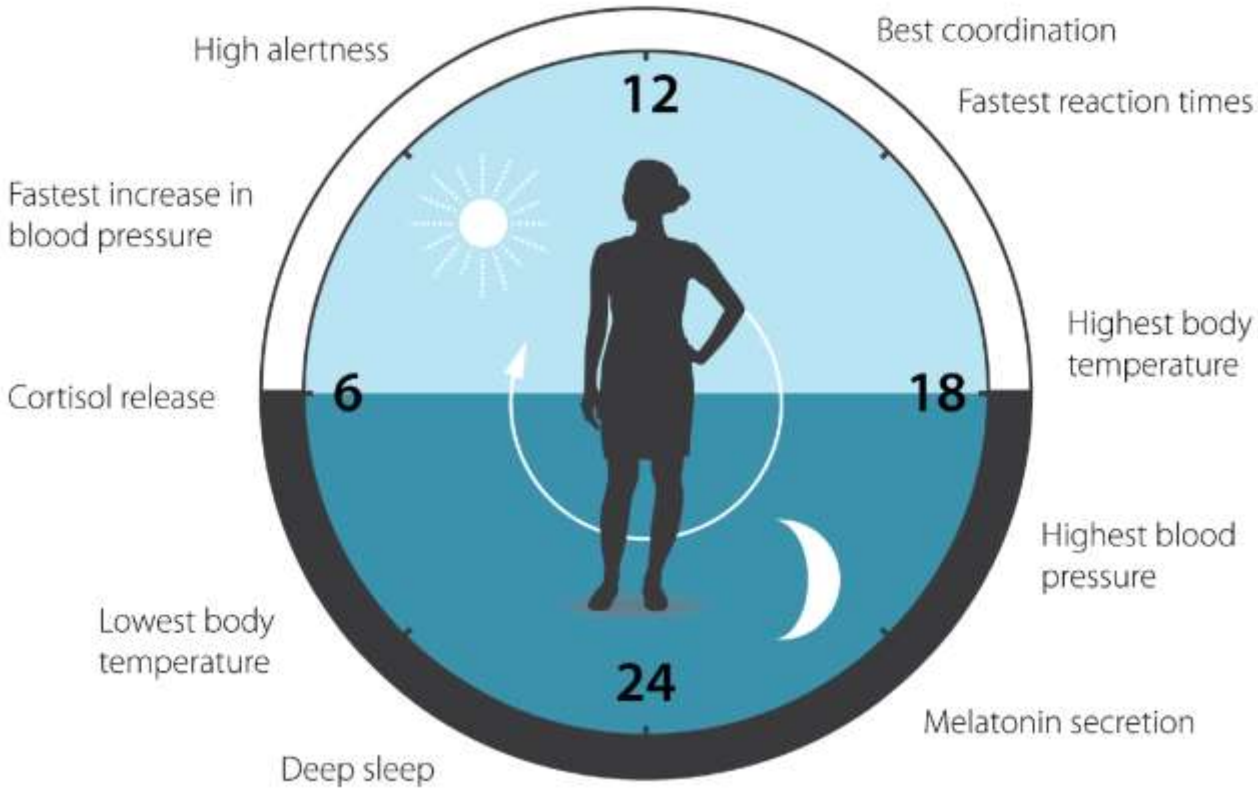
What time is it ?

Ilka Maria Axmann
Synthetic Microbiology
Heinrich Heine University Düsseldorf

advantages of biological oscillations:

- ✓ temporal organization
- ✓ spatial organisation
- ✓ prediction of repetitive events
- ✓ efficiency
- ✓ precision of control

P. E. Papp Progress in Neurobiology 1986



www.nobelprize.org/nobel_prizes/medicine/laureates/2017/

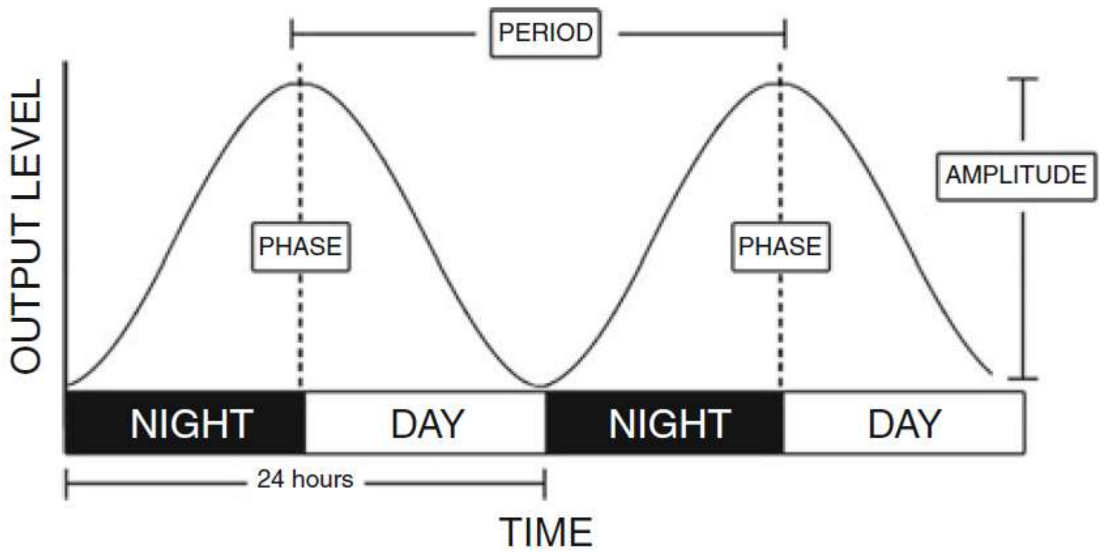
… circadian clock

circa = around

dies = day

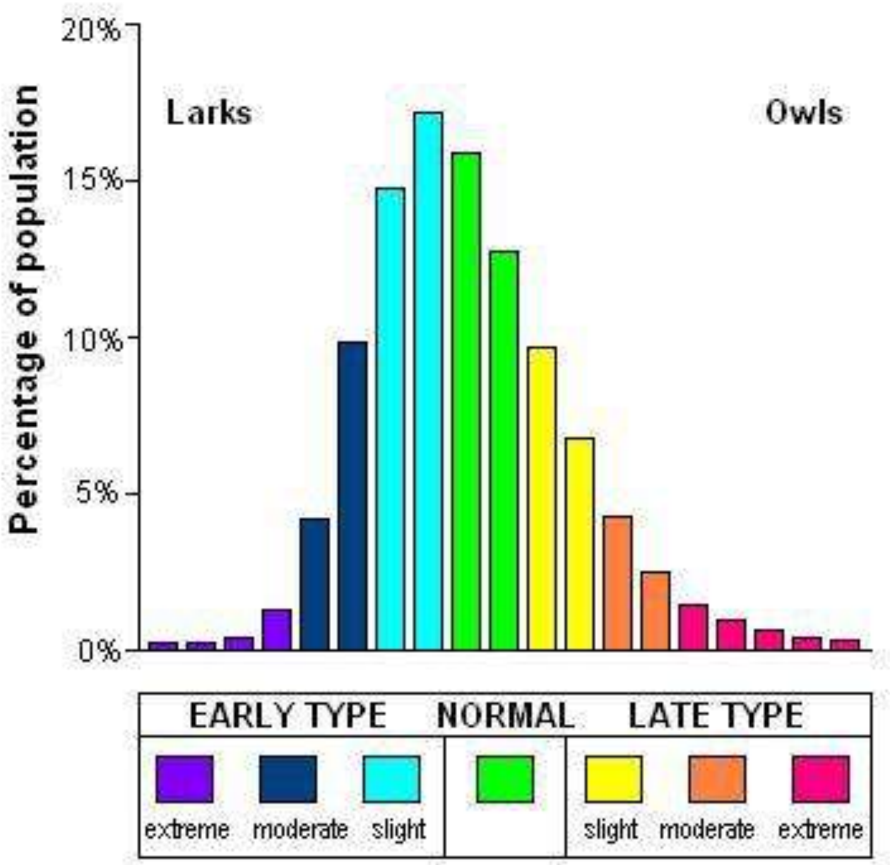
endogenous oscillator
period length of about 24 hours
temperature-compensated

… common fundamental properties

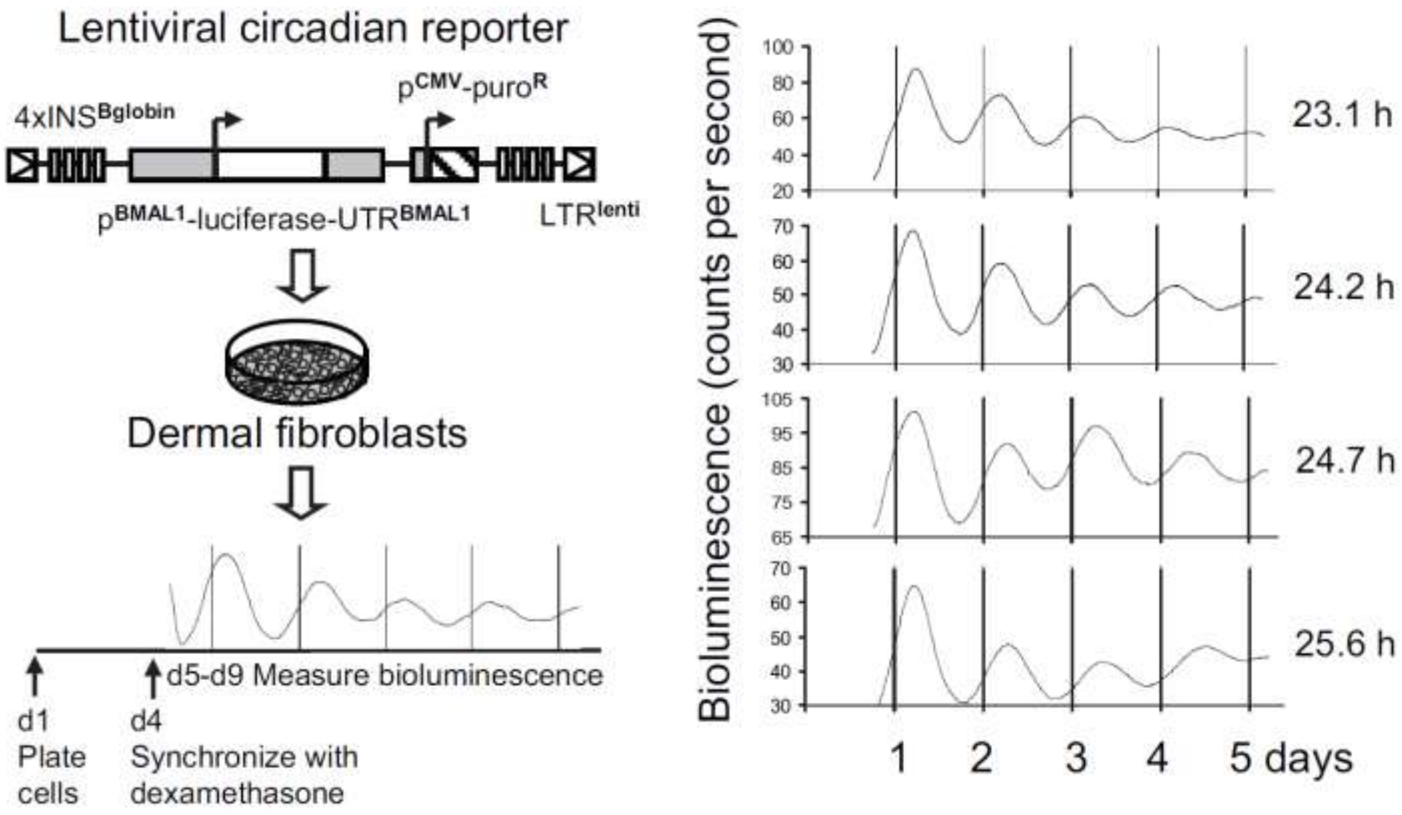


Silver & Rainbow 2013

… distribution of circadian period lengths

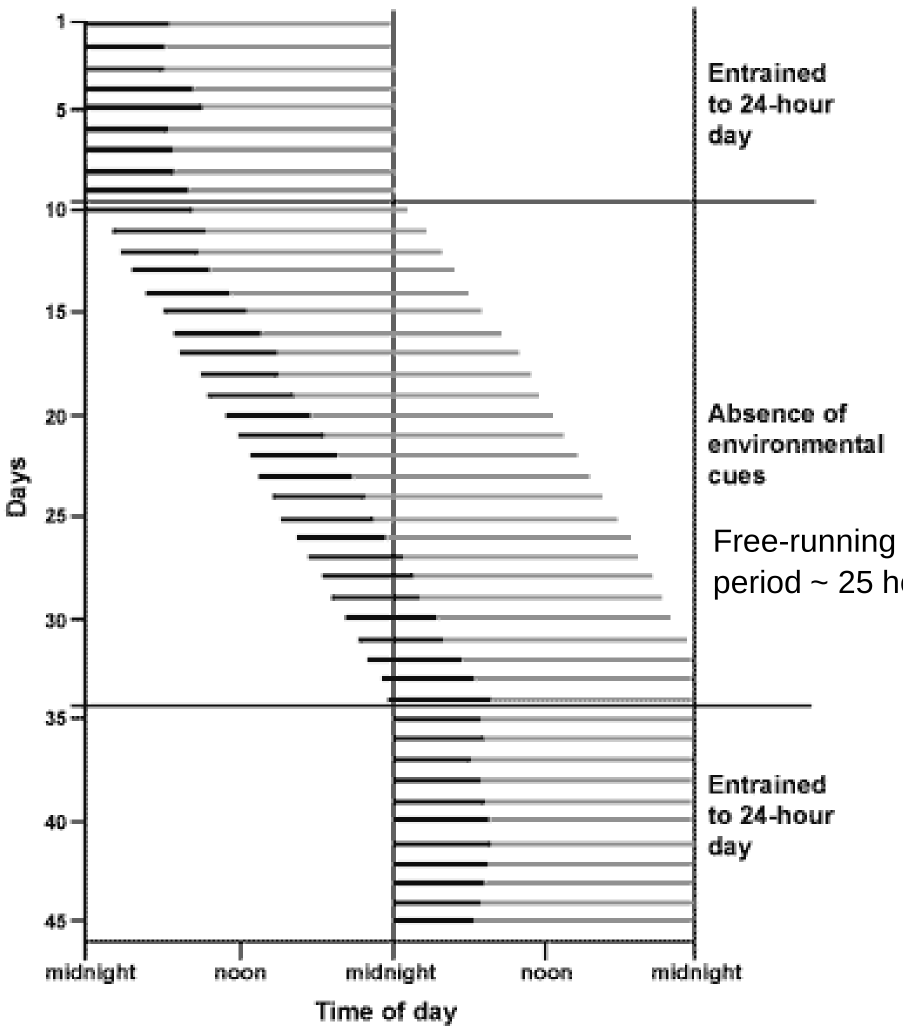


… free-run



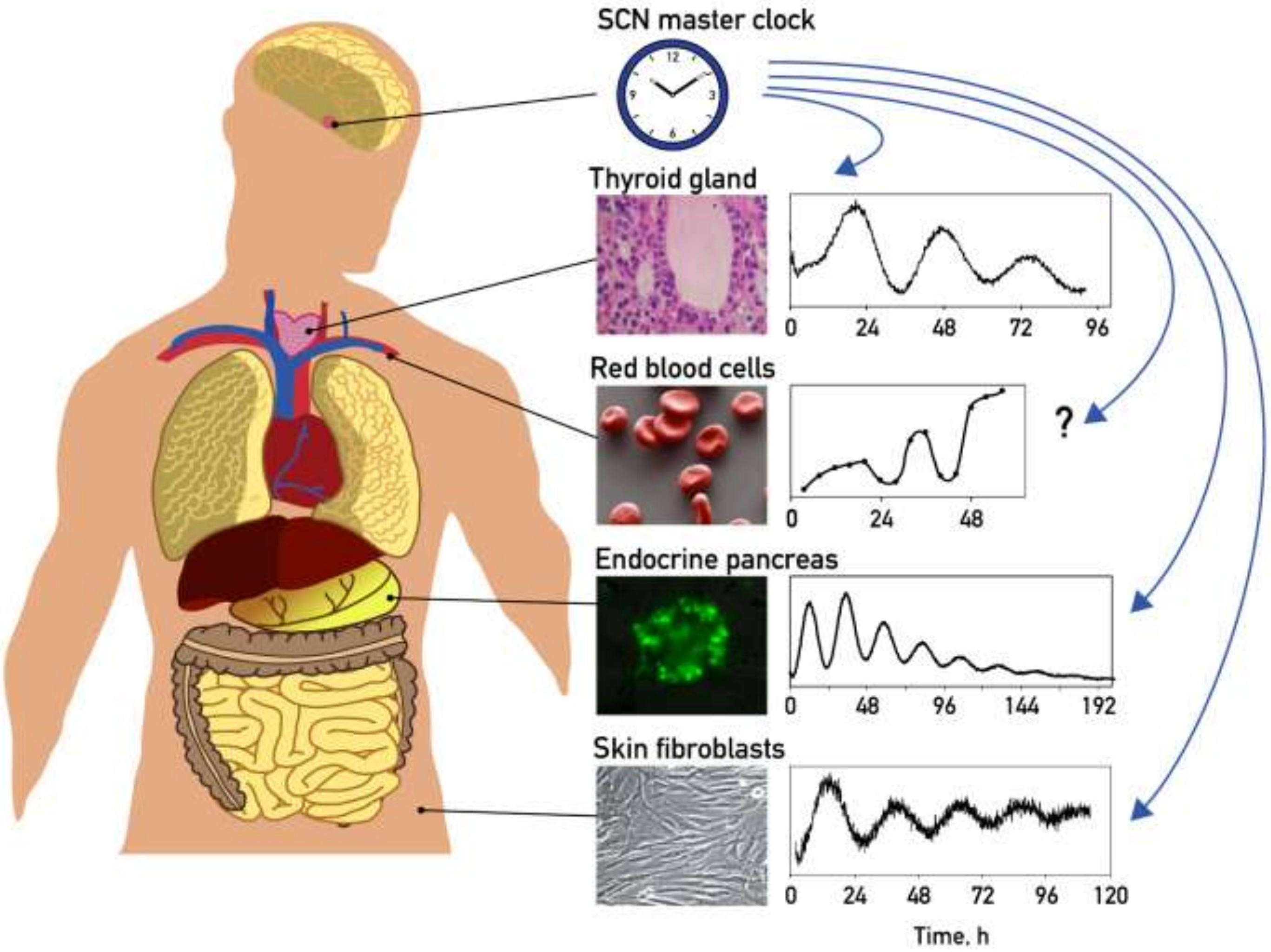
Brown Westermark Herzel Kramer et al. PNAS 2008

… entrainment



Free-running period ~ 25 hours

… central and peripheral clocks



Dibner & Schibler Journal of Internal Medicine 2015

… genetic code and information flow

DNA

↓

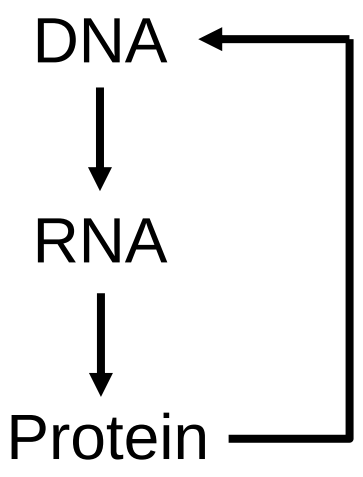
RNA

↓

Protein

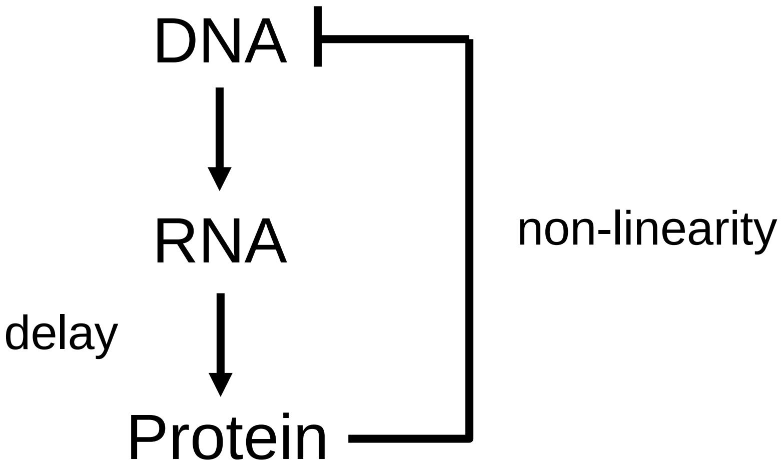
American biologist James Watson and English physicist Francis Crick 1950s

… gene regulation

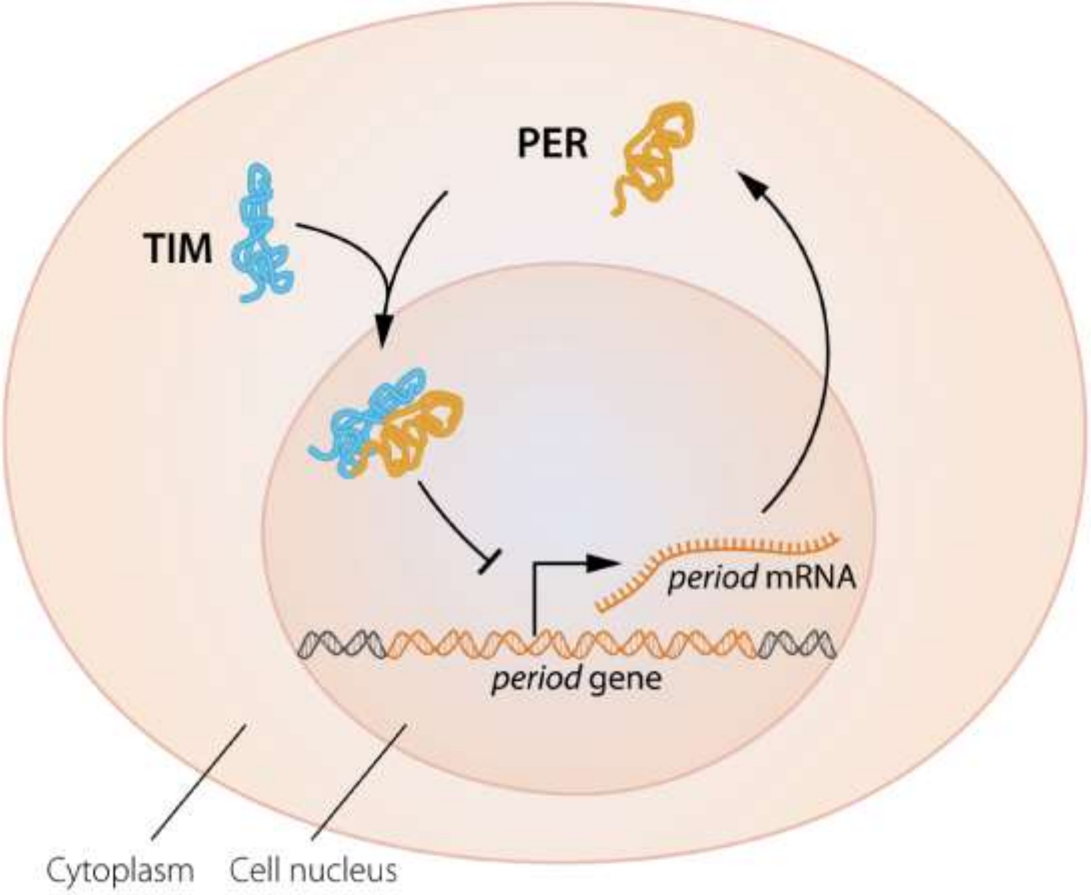


e.g. lac operon François Jacob and Jacques Monod 1961

… negative feedback loop

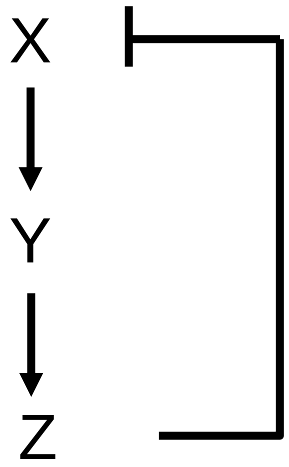


… circadian rhythm in fruit flies



www.nobelprize.org/nobel_prizes/medicine/laureates/2017/

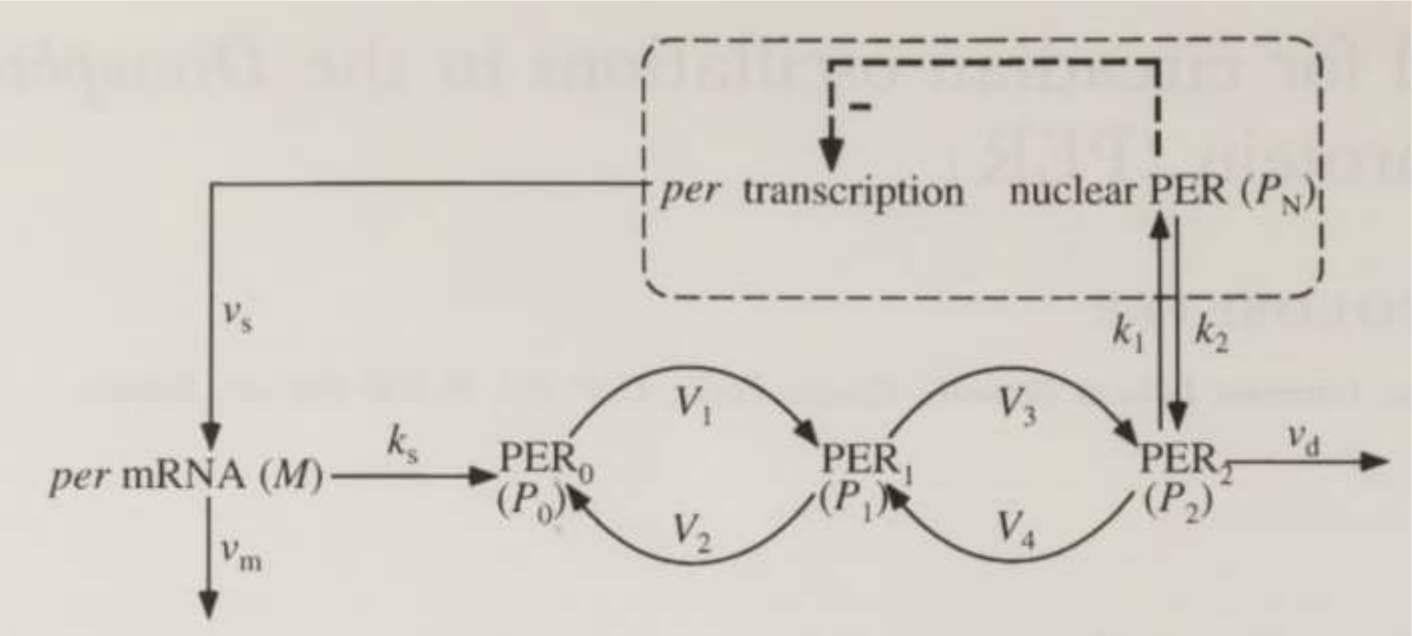
… non-linearity



$$\frac{dX}{dt} = \frac{k_1}{Z^n + 1} - k_4 X$$

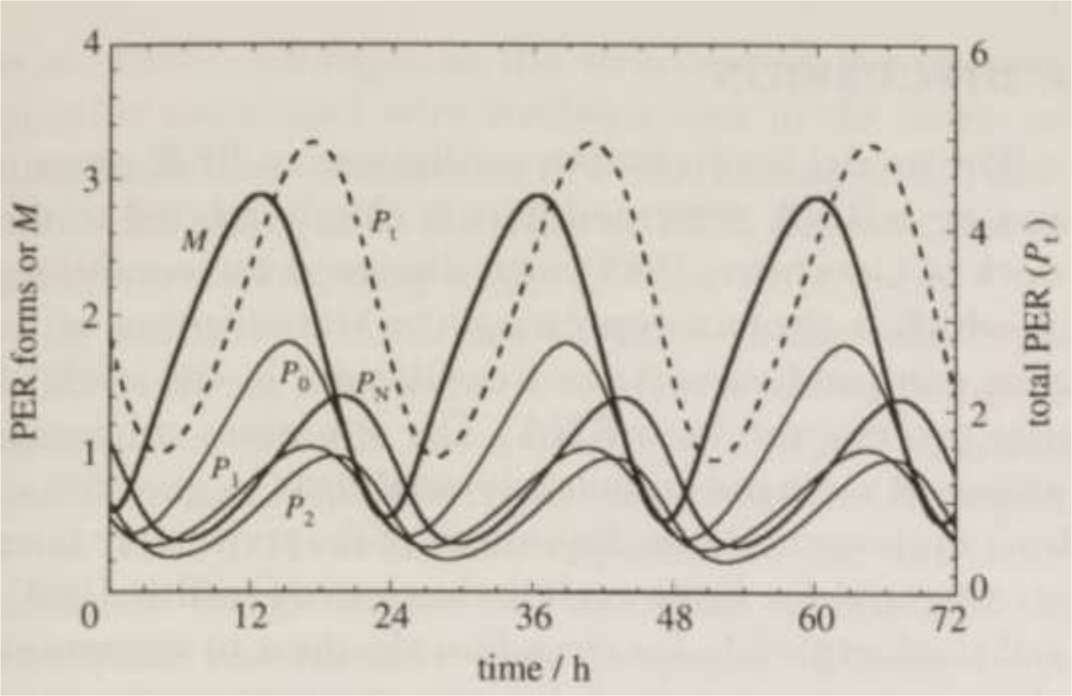
Goodwin 1963

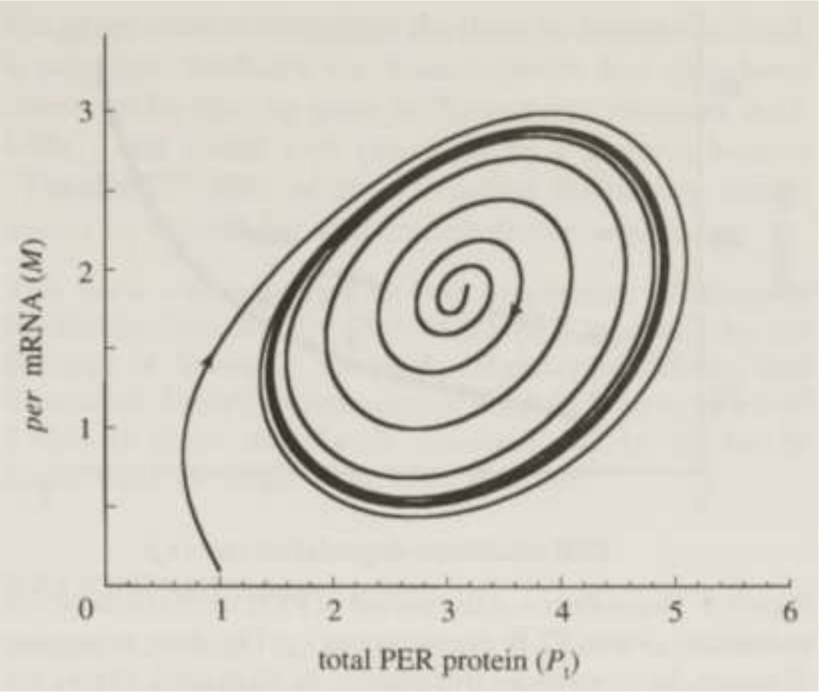
… transcription-translation feedback loop (TTFL)



Goldbeter Proc Biol Sci 1995

… sustained oscillations





Goldbeter Proc Biol Sci 1995

… sustained oscillations in cyanobacteria

J. Phycol. **25**, 183–186 (1989)

NOTE

A CIRCADIAN RHYTHM IN CELL DIVISION IN A PROKARYOTE, THE CYANOBACTERIUM *SYNECHOCOCCUS* WH7803[1]

Beatrice M. Sweeney[2] *and M. Beatriz Borgese*[3]

Department of Biological Sciences, University of California, Santa Barbara, California 93106

ABSTRACT

Circadian rhythms are common in eukaryotes, but the several claimed cases in prokaryotes are all open to alternative interpretation. We report here a clearcut circadian rhythm in cell division in a marine Synechococcus *sp. strain WH7803, under conditions where the generation time is longer than one day, that is entrained by a light–dark cycle, and that persists for at least four cycles in continuous light (2 μE·m⁻²·s⁻¹) and constant temperature (22, 20 or 16° C) with a maximum in dividing cells at about 24 h intervals. Thus, the prokaryote,* Synechococcus, *satisfies the criteria for the possession of a true temperature-compensated circadian clock. Were the existence of such a rhythm confirmed, current hypotheses that intracellular compartments are required for circadian timing may require modification.*

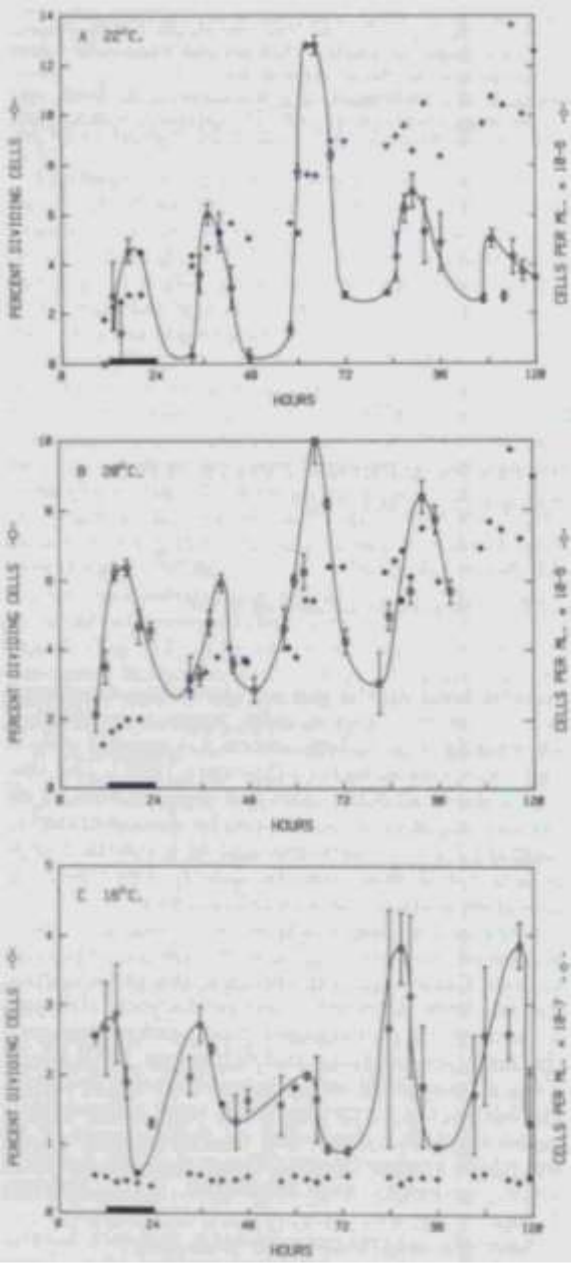
Key index words: *cell division rhythm; circadian clock; cyanobacterium; prokaryote;* Synechococcus *WH7803; temperature compensation*

become hard. In the marine plank
lates, photosynthesis is most acti
whereas light as bioluminescence i
night when it can be seen (Sweeney
ing of these activities does not de
the day–night cycle; it can also be
constant light or darkness and at
perature and thus must be innate
sponsible is known as a circadian clock.

Circadian timing has now been found in many diverse eukaryotes ranging from unicellular protists to man. Curiously, until recently, this phenomenon had not been detected in any prokaryote, although a number of investigators have looked for such rhythms (see Hastings and Schweiger 1976:49–50 for a discussion of unpublished negative findings). Models of the circadian clock were consequently constructed that depended on the presence of intracellular compartments (Njus et al. 1974, Sweeney 1974, Schweiger and Schweiger 1977, Sweeney

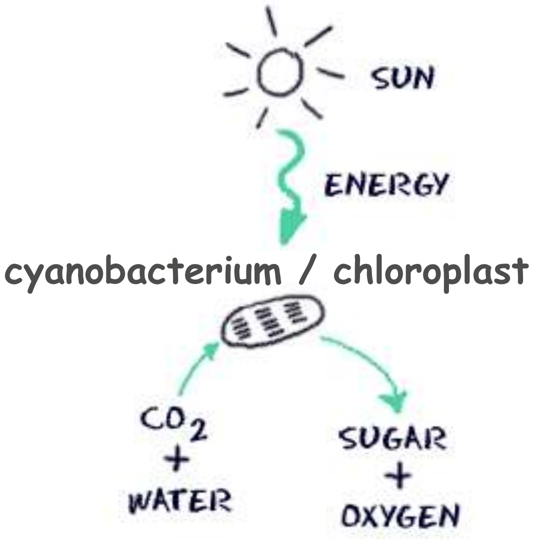
… entrainable by light-dark cycle
… persisting in continuous light

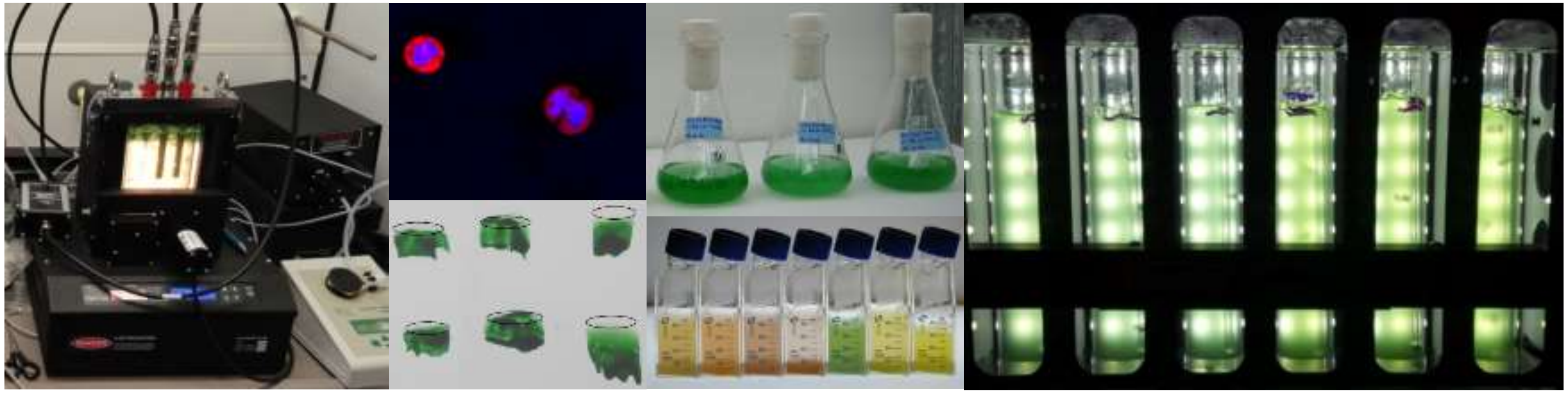
- ✓ true circadian clock
- ✓ without compartments



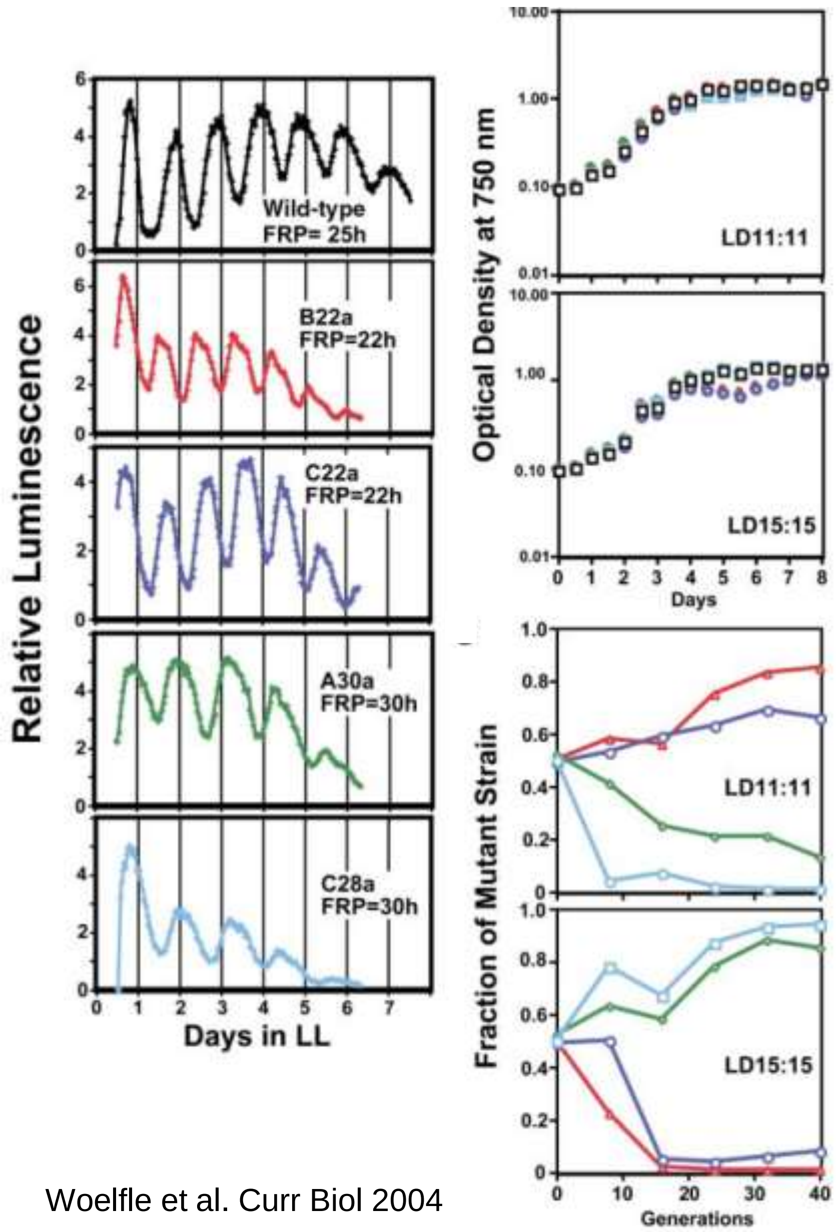
Life on Earth is driven by the power of oxygenic photosynthesis

transforming solar into chemical energy.



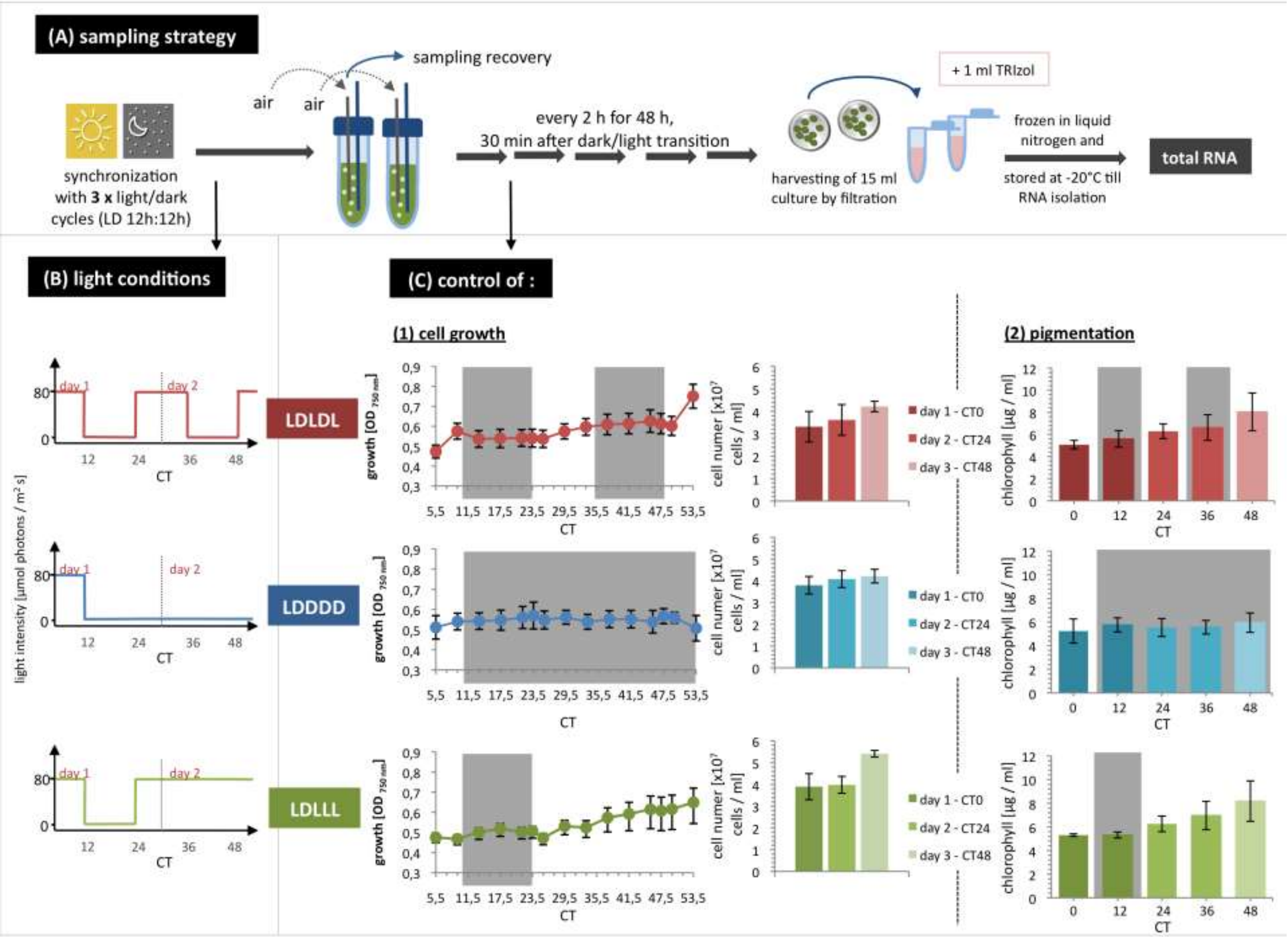


… fitness advantage – extrinsic vs. intrinsic adaptive value



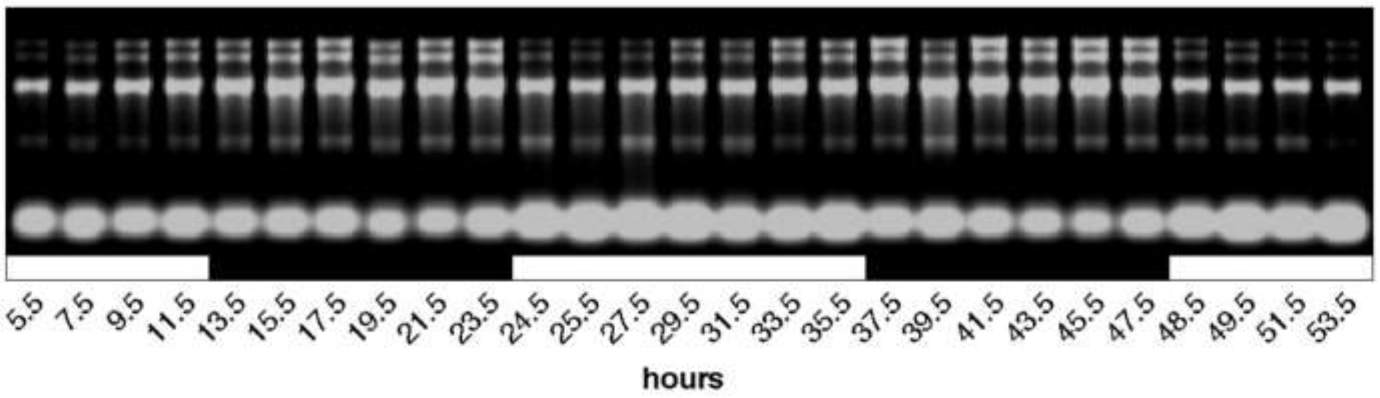
Woelfle et al. Curr Biol 2004

Do cyanobacteria sleep at night ?



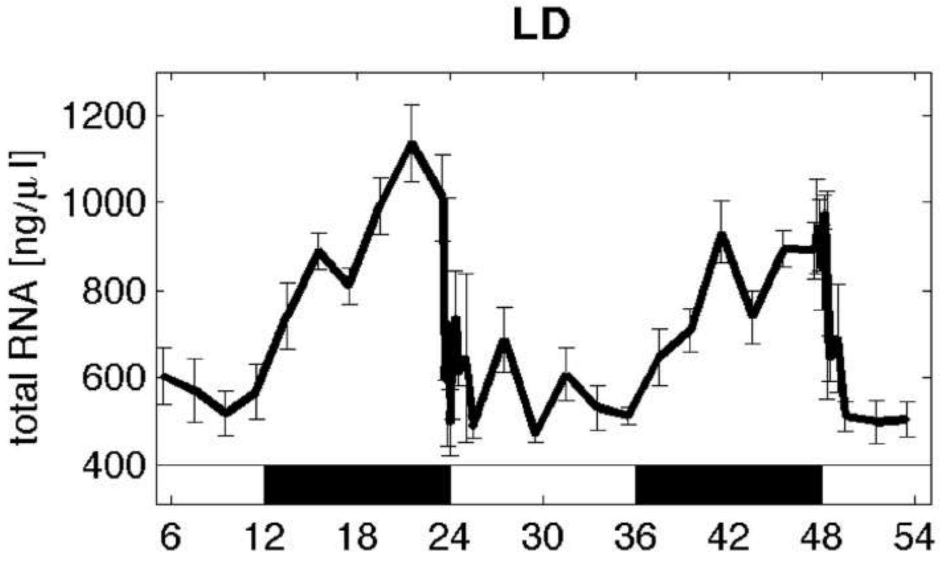
(A) sampling strategy
sampling recovery
air
air
+ 1 ml TRIzol
every 2 h for 48 h,
30 min after dark/light transition
synchronization
with 3 x light/dark
cycles (LD 12h:12h)
harvesting of 15 ml
culture by filtration
frozen in liquid
nitrogen and
stored at -20°C till
RNA isolation
total RNA
(B) light conditions
(C) control of :
(1) cell growth
(2) pigmentation
light intensity [µmol photons / m² s]
day 1
day 2
CT
LDLDL
LDDDD
LDLLL
growth [OD 750 nm]
cell numer [x10⁷ cells / ml]
day 1 - CT0
day 2 - CT24
day 3 - CT48
chlorophyll [µg / ml]

… daily changes in total RNA and rRNA in *Synechocystis*



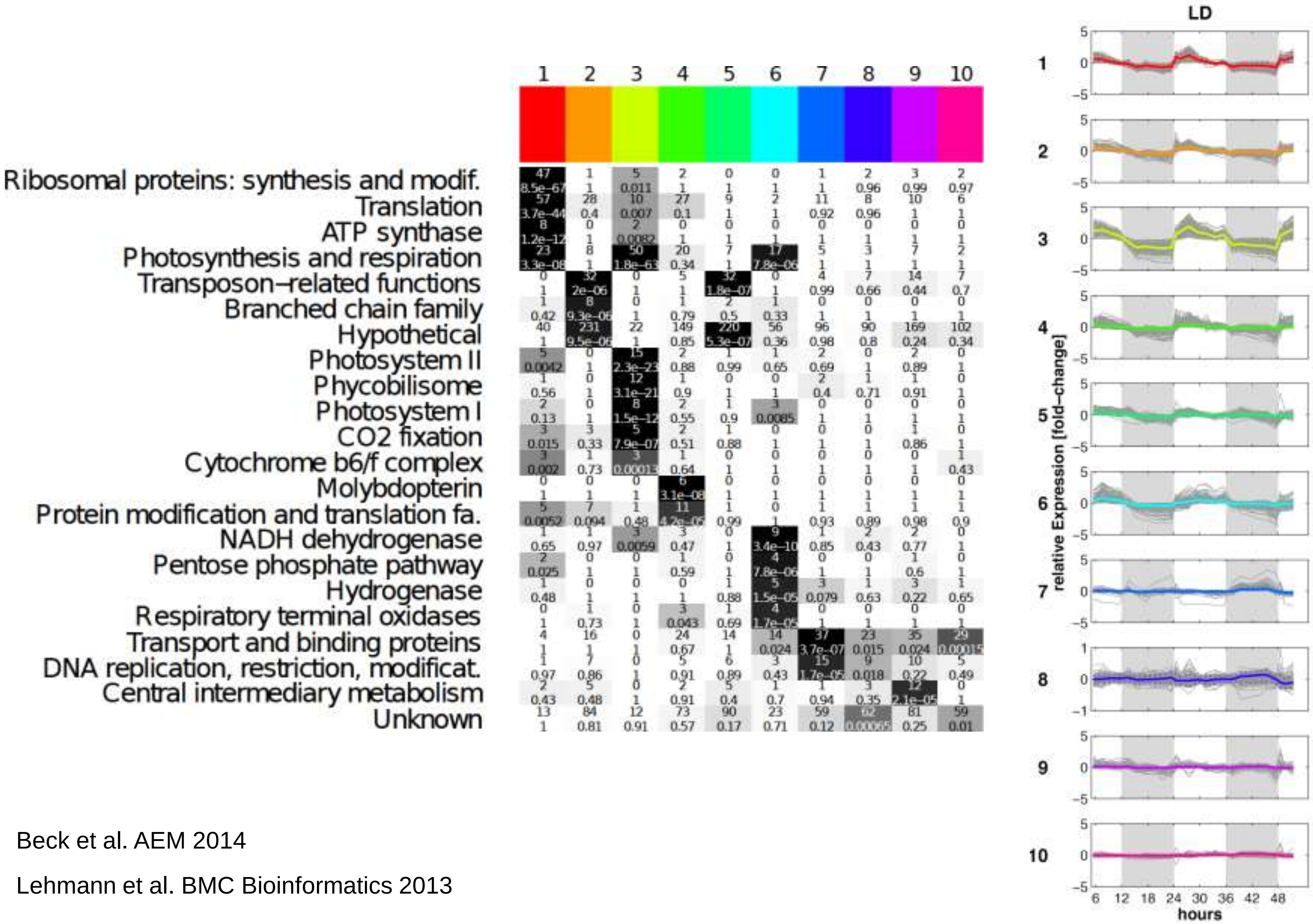
Beck et al. AEM 2014

… daily changes in total RNA and rRNA in *Synechocystis*



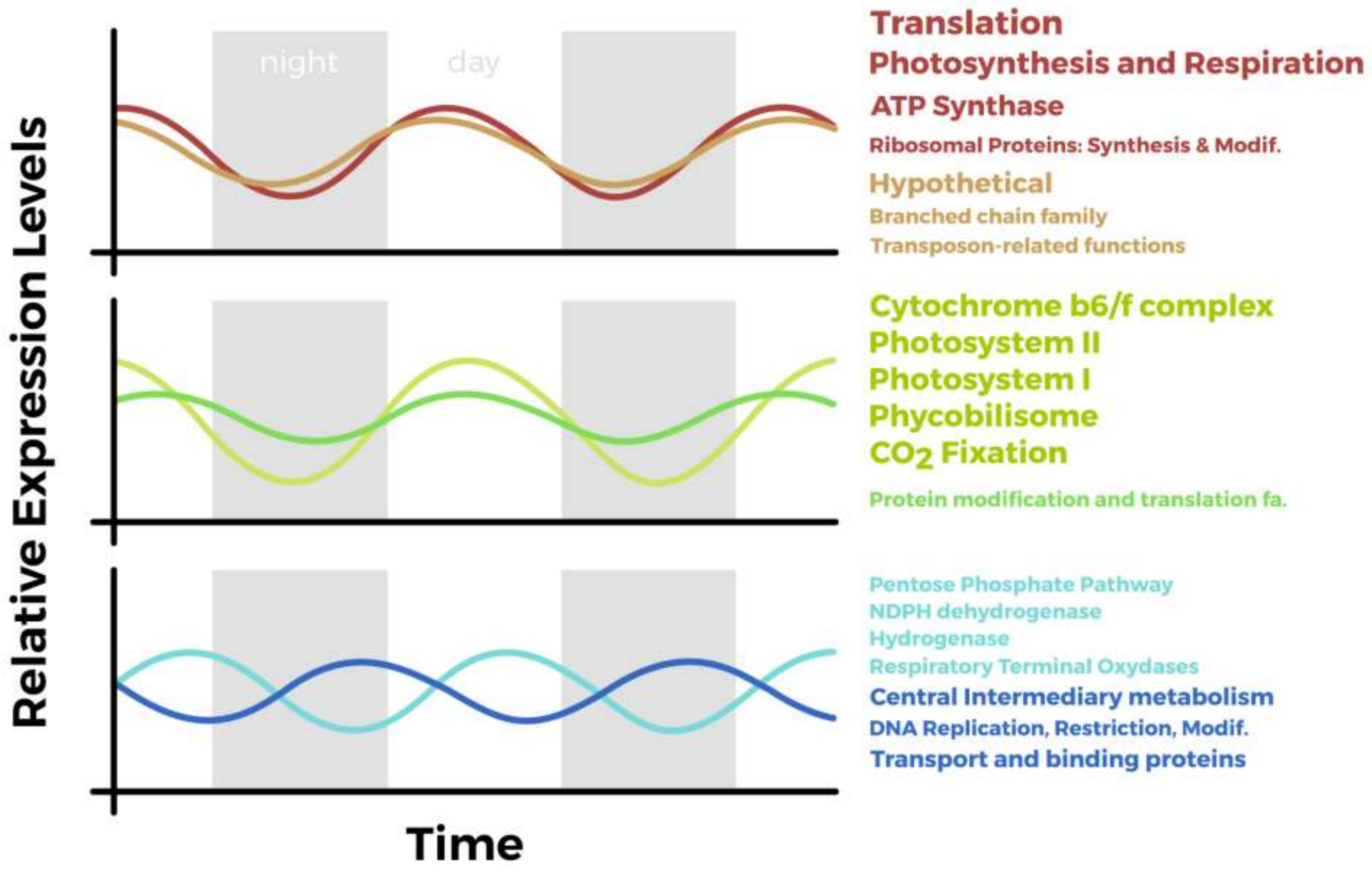
Beck et al. AEM 2014

… daily program of a photosynthetic cell

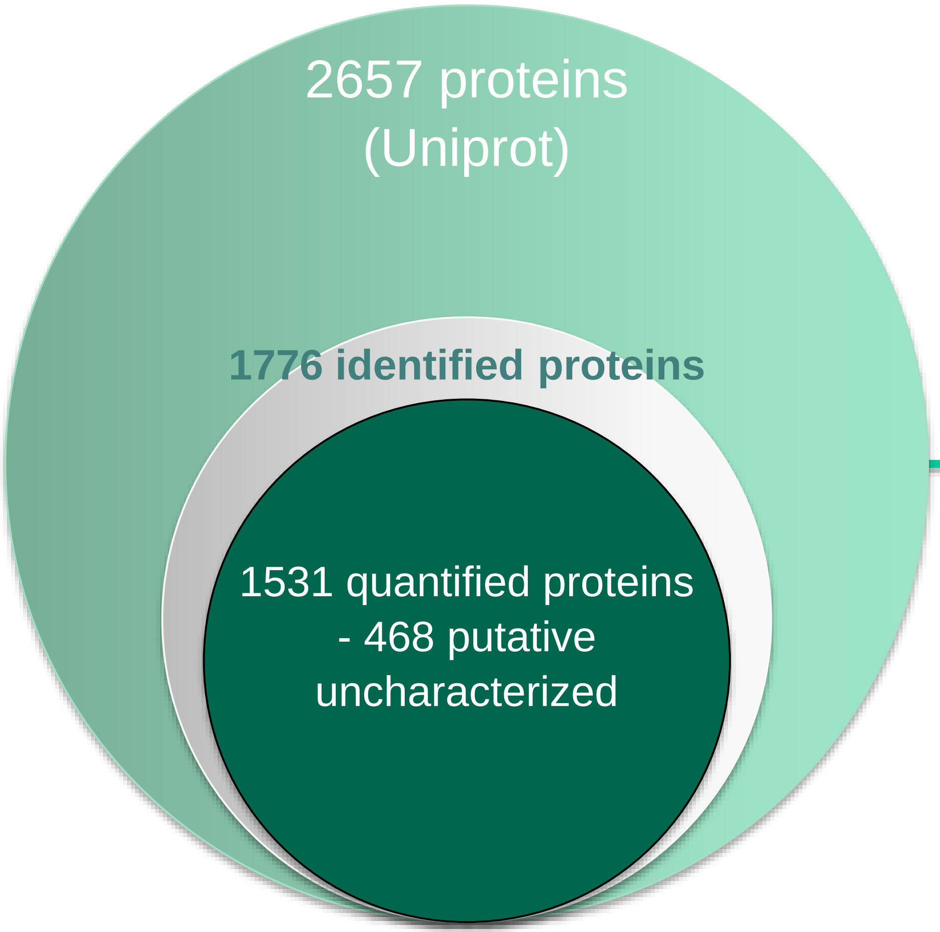


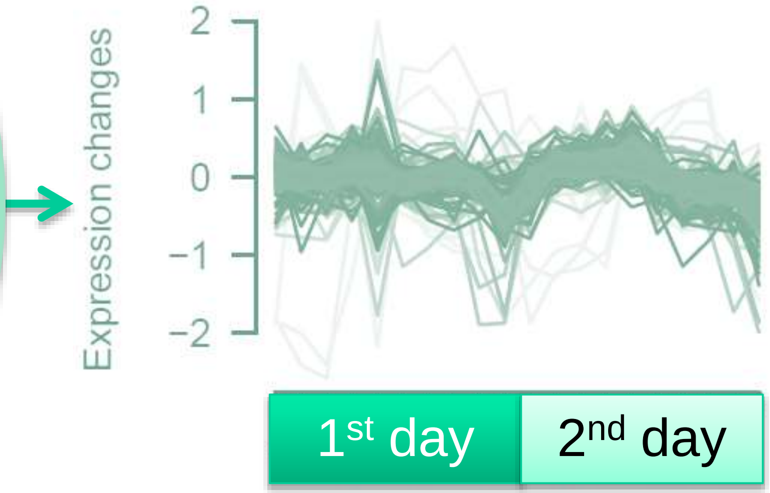
Beck et al. AEM 2014

Lehmann et al. BMC Bioinformatics 2013


night
day
Relative Expression Levels
Time
Translation
Photosynthesis and Respiration
ATP Synthase
Ribosomal Proteins: Synthesis & Modif.
Hypothetical
Branched chain family
Transposon-related functions
Cytochrome b6/f complex
Photosystem II
Photosystem I
Phycobilisome
CO_2 Fixation
Protein modification and translation fa.
Pentose Phosphate Pathway
NDPH dehydrogenase
Hydrogenase
Respiratory Terminal Oxydases
Central Intermediary metabolism
DNA Replication, Restriction, Modif.
Transport and binding proteins

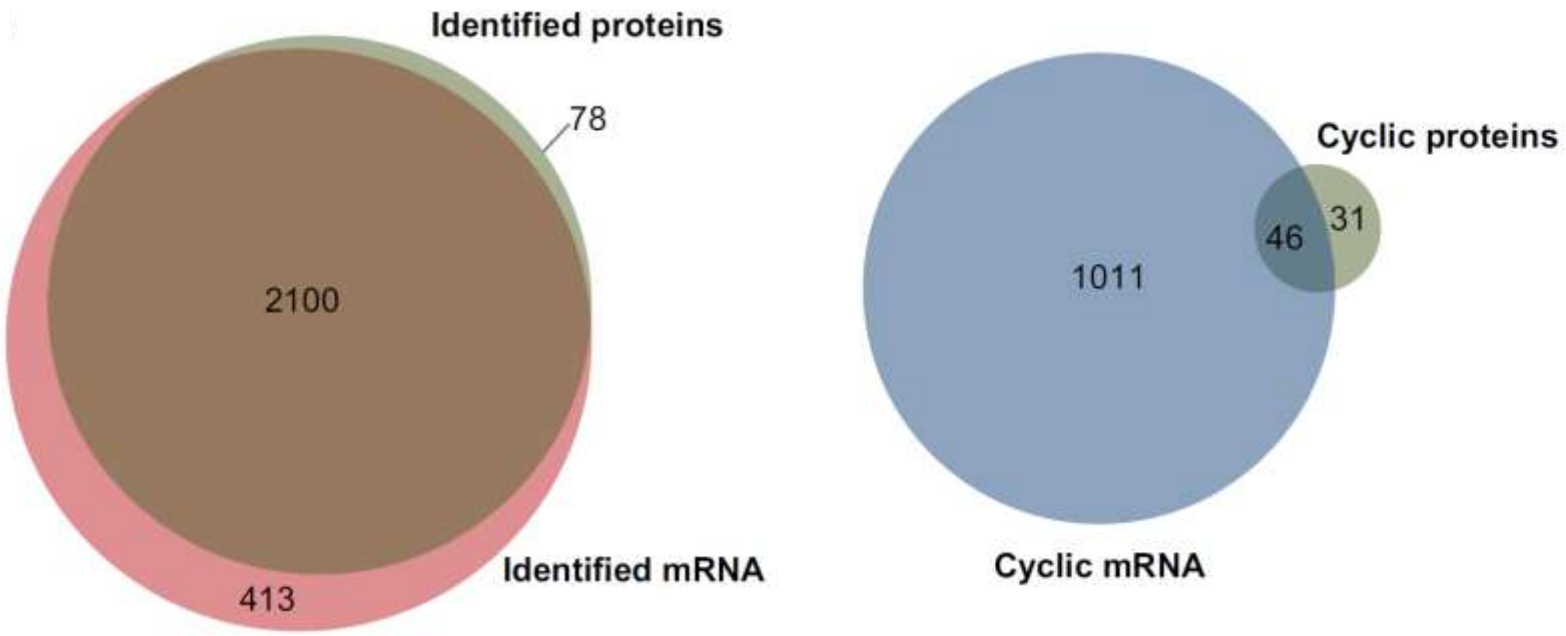
… cyclic proteome of *Synechococcus elongatus*





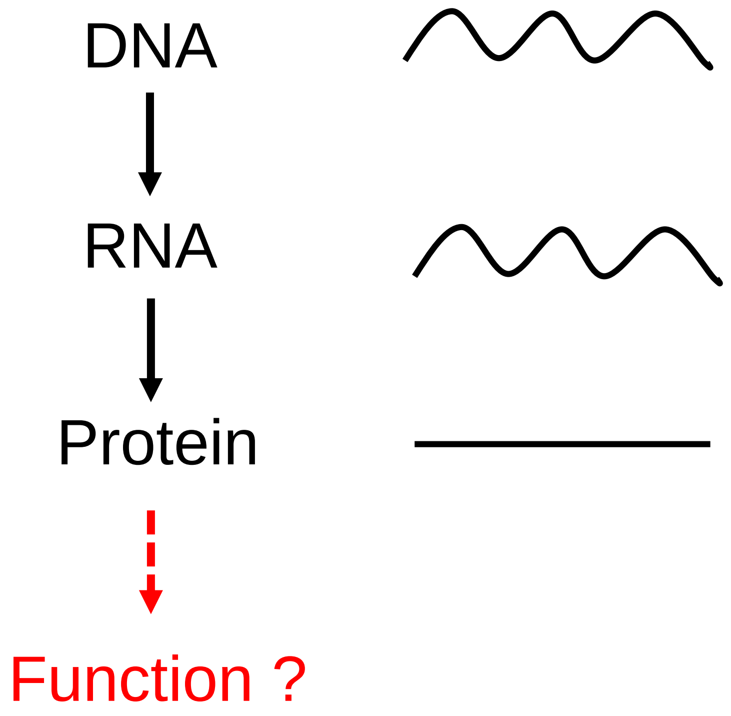
Figures kindly provided by Ana Guerreiro

… cyclic proteome … is minimal

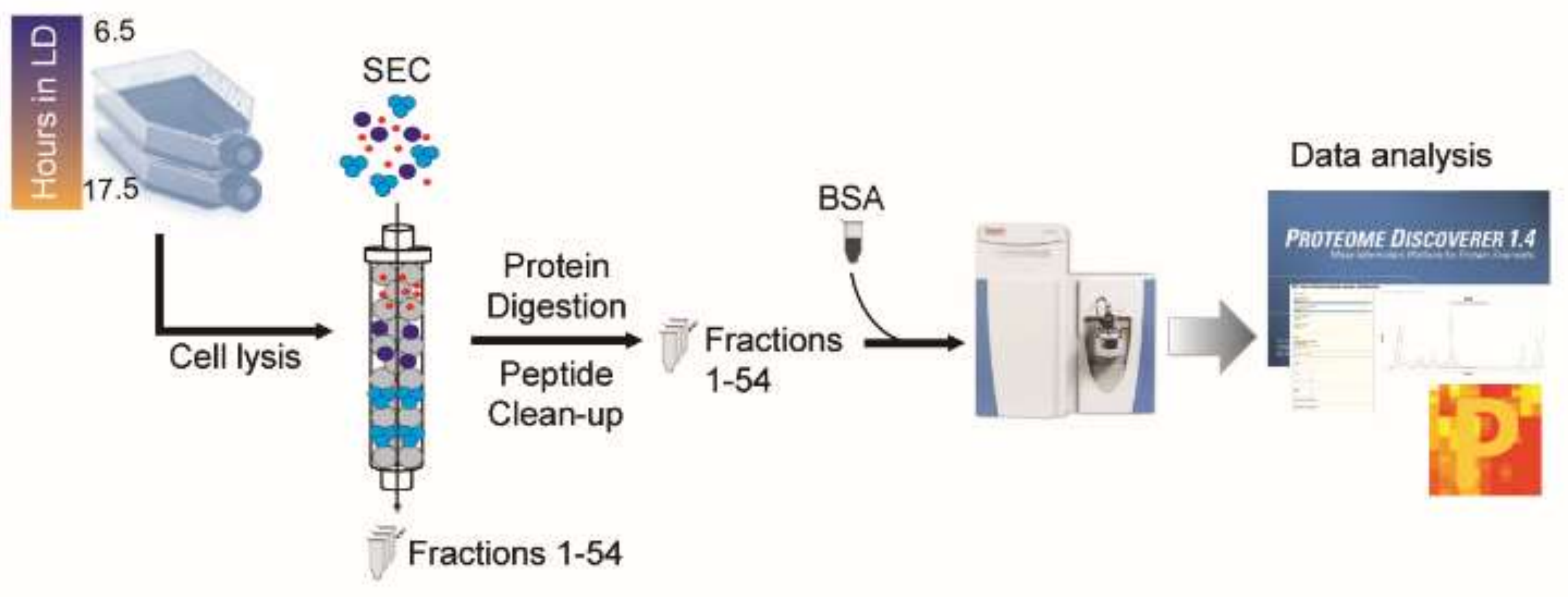


Guerreiro et al. Mol Cell Proteomics 2014

… information flow

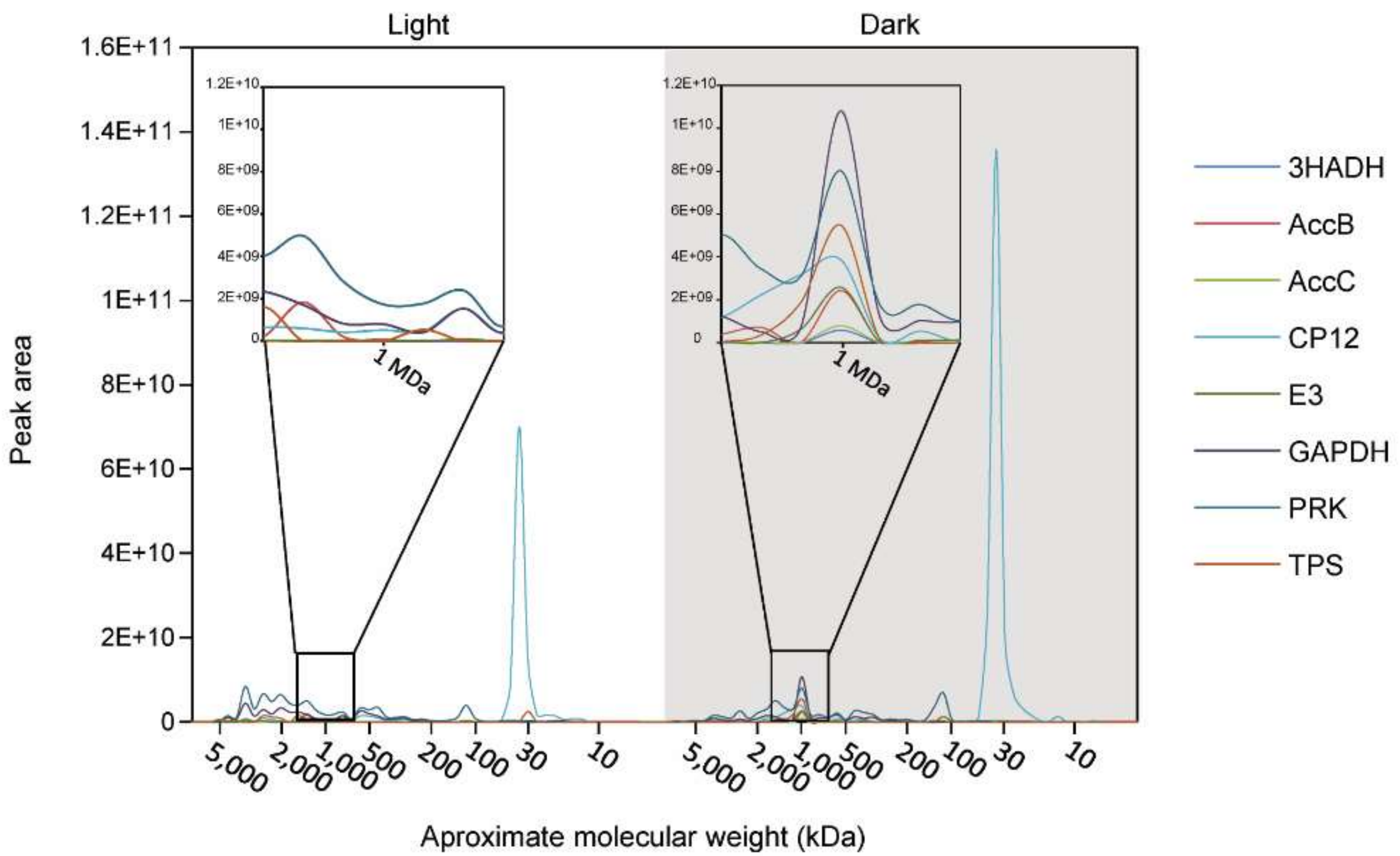


… dynamics of multi-protein complexes



Guerreiro et al. J Proteomics. 2016

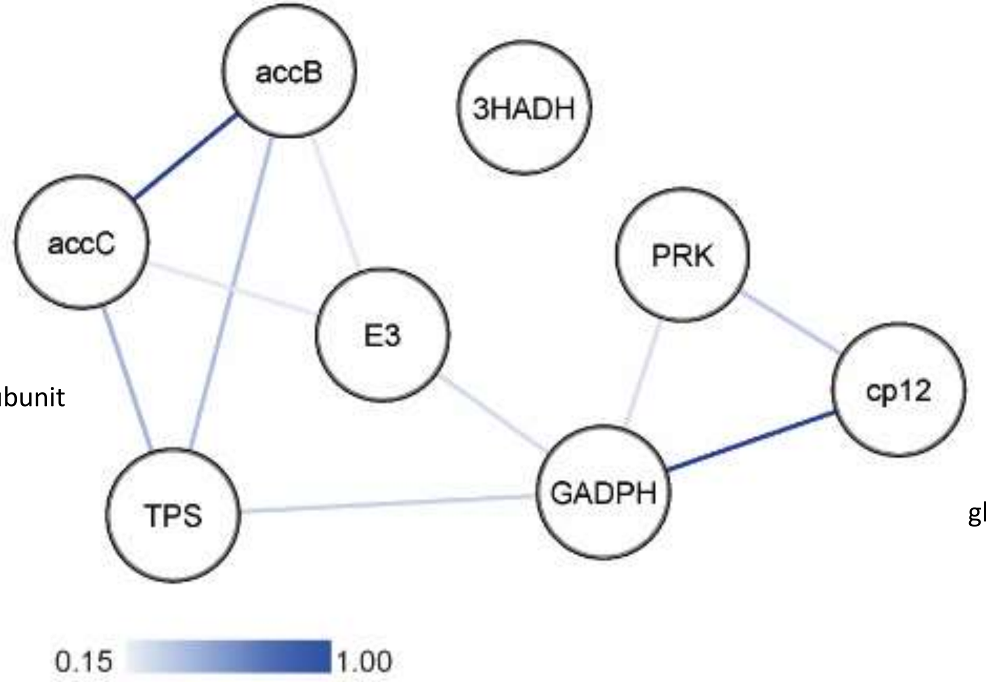
… dynamics of metabolic pathway proteins



Guerreiro et al. J Proteomics. 2016

… 1 MDa complex linking
glycolysis, pyruvate metabolism and carbon fixation

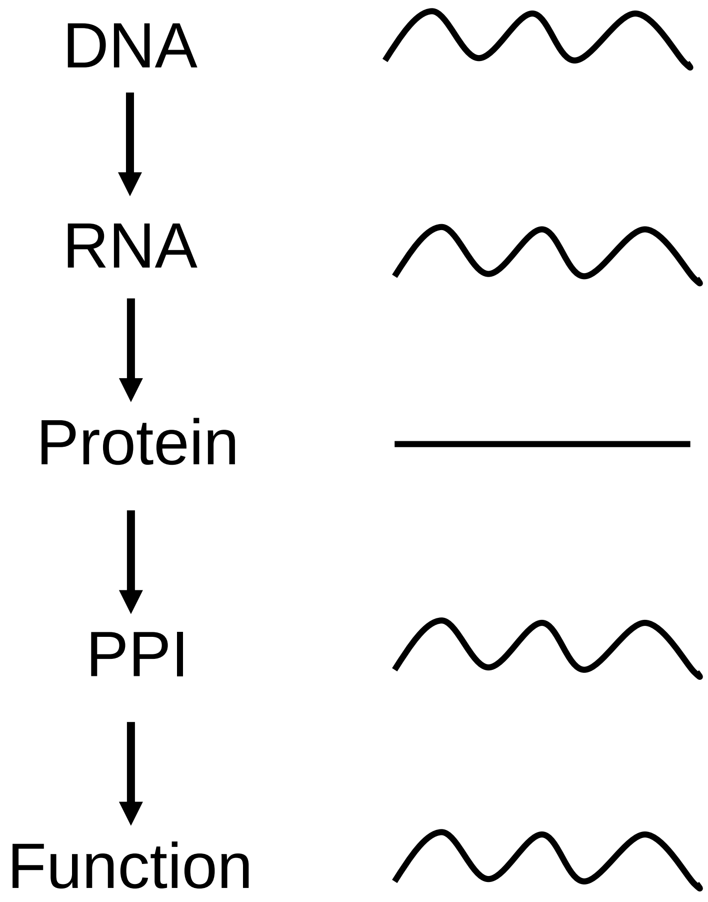
biotin carboxyl carrier protein (AccB)
acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha/biotin carboxylase (AccC)
dihydroplipoyl dehydrogenase (E3)
thiamine-phosphate synthase (TPS)

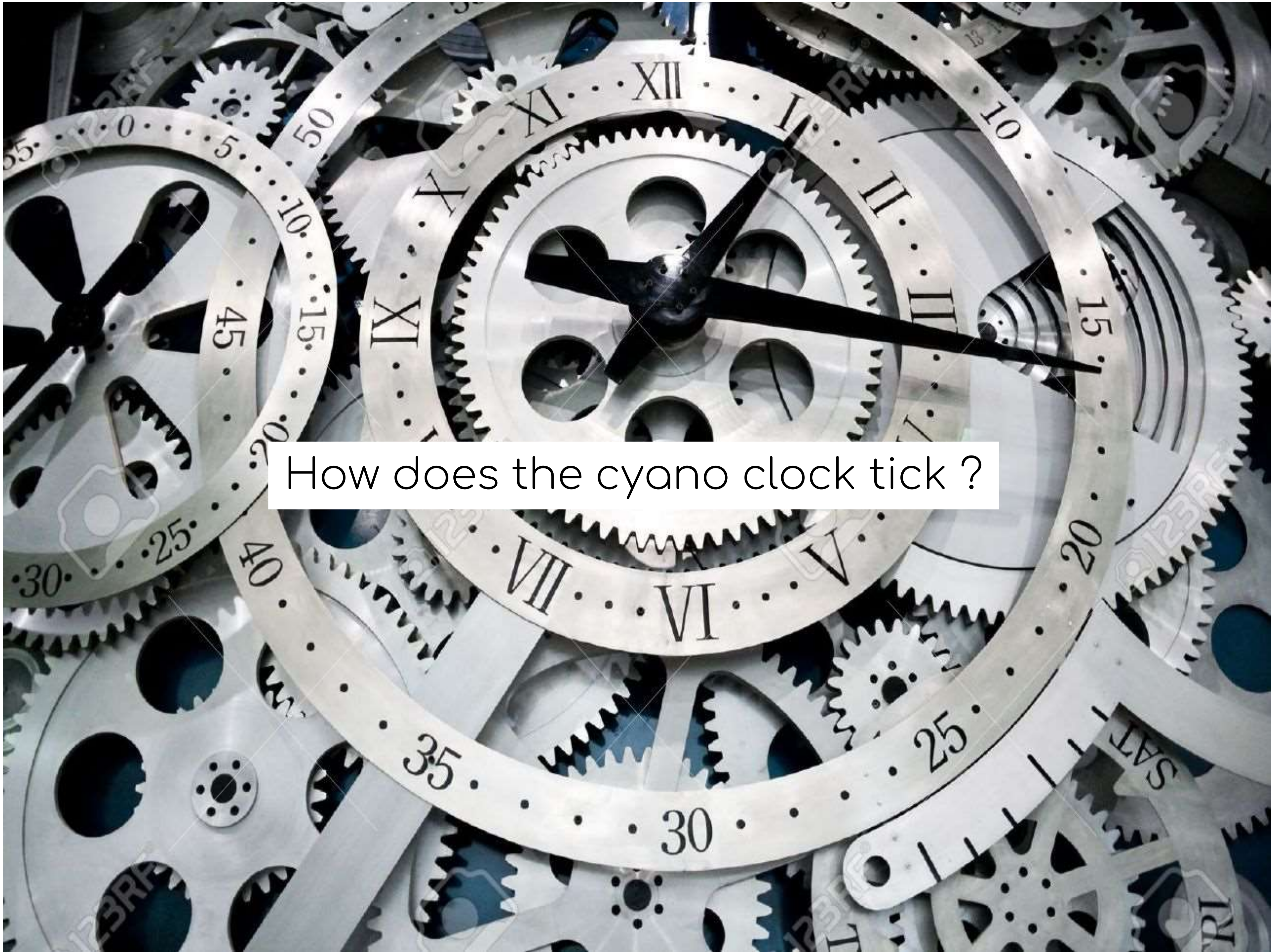


3-hidroxyacid dehydrogenase (3HADH)
phosphoribulokinase (Prk)
dihydroplipoyl dehydrogenase (E3)
linker CP12
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Guerreiro et al. J Proteomics. 2016

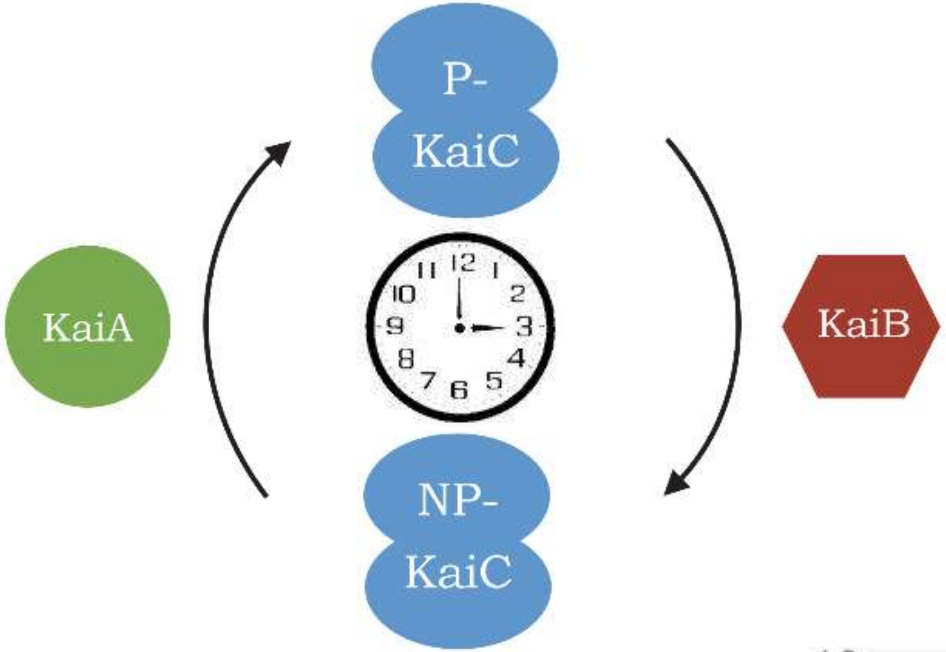
… information flow

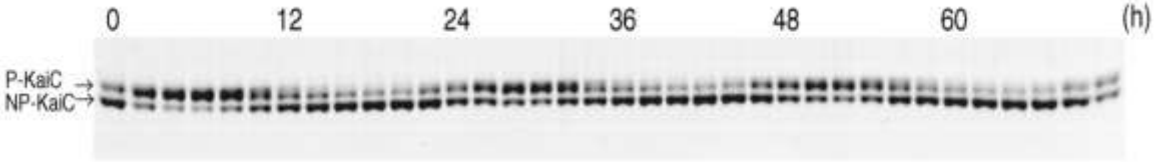


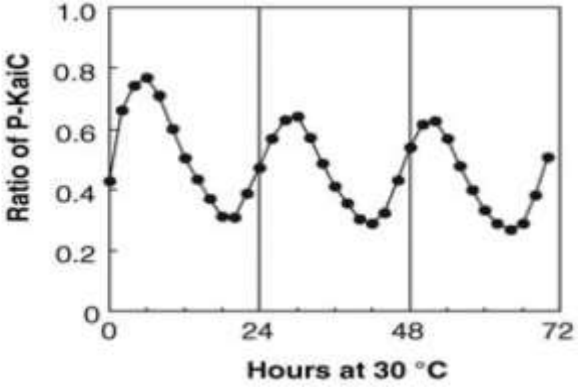


How does the cyano clock tick ?

… 3-protein clock of *Synechococcus elongatus*

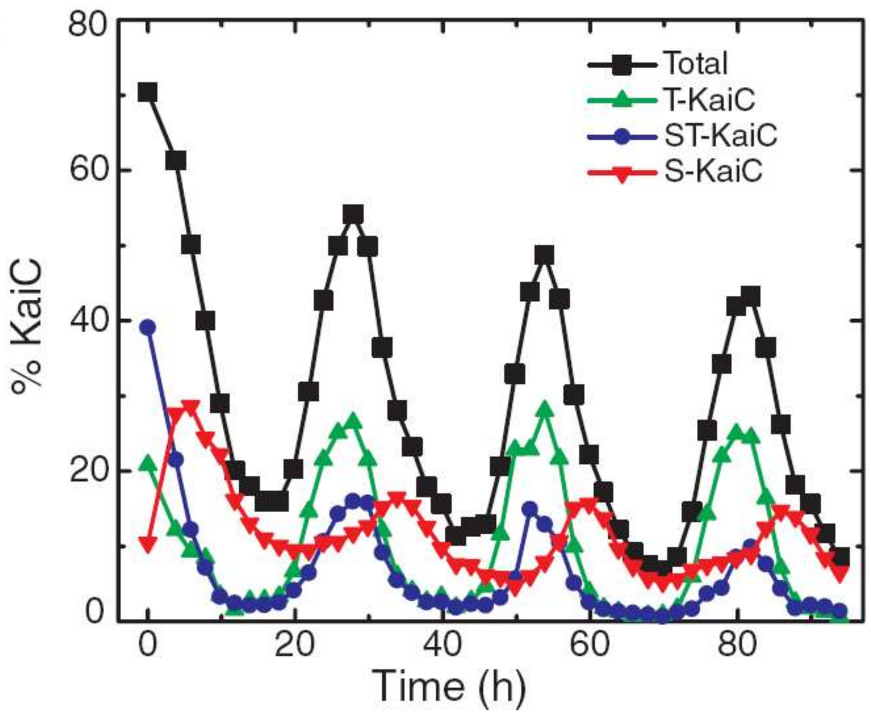


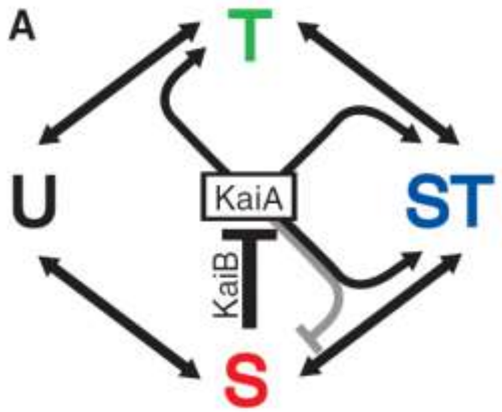




Nakajima et al., 2005

… phosphorylation of KaiC hexamers is ordered



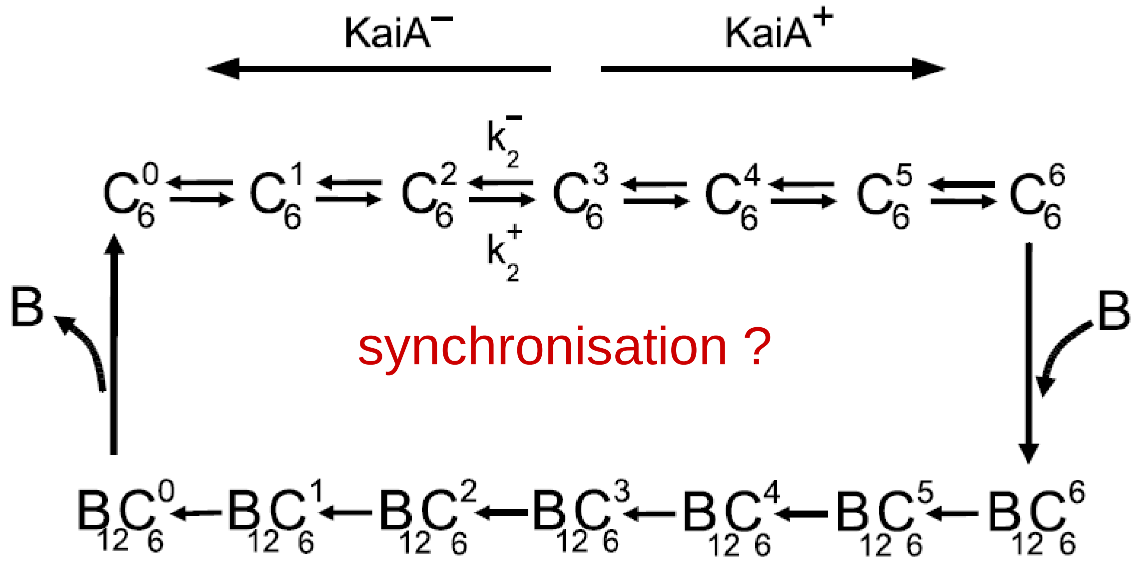


Rust et al., 2007, Science

… modeling a core reaction network

model assumptions:

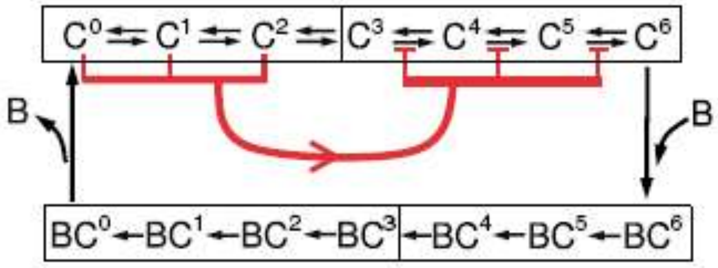
C^0 and C^6 = oscillating states of KaiC, low P and high P
C_6 = KaiC hexamers: low P and high P depend on actual reaction rates
k = phosphorylation rates depend on KaiA level

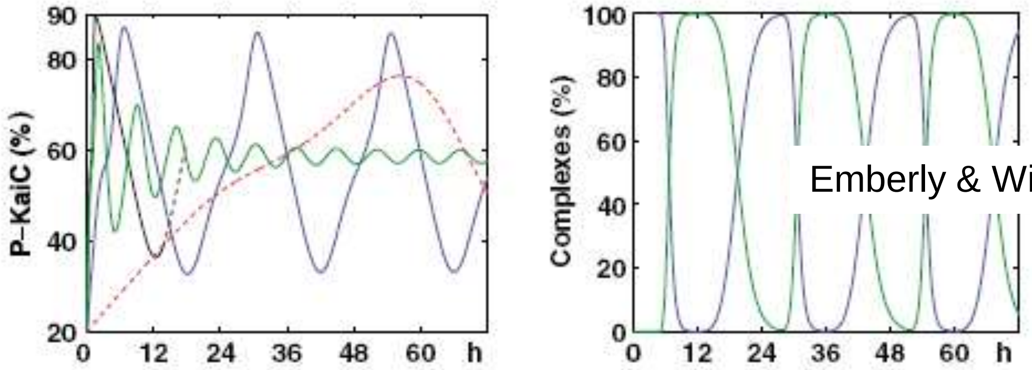


C_6 highest P-state binds six KaiB stably
$B_{12}C_6$ dephosphorylates and releases KaiB dimers in the lowest phosphorylation state

… systematic scan of reaction networks having a feedback loop

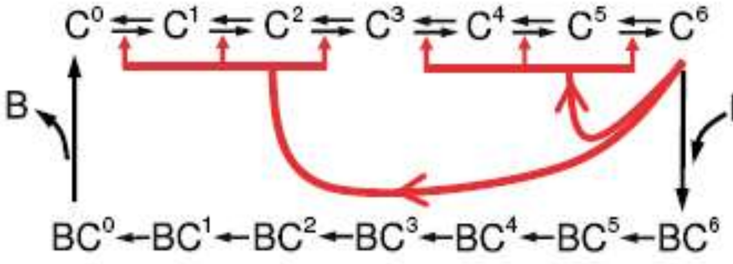
negative feeback (monomer exchange)

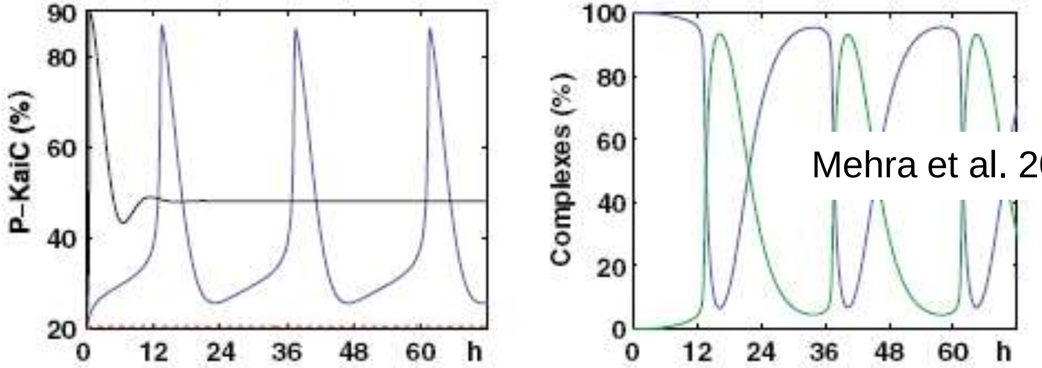




Emberly & Wingreen 2006

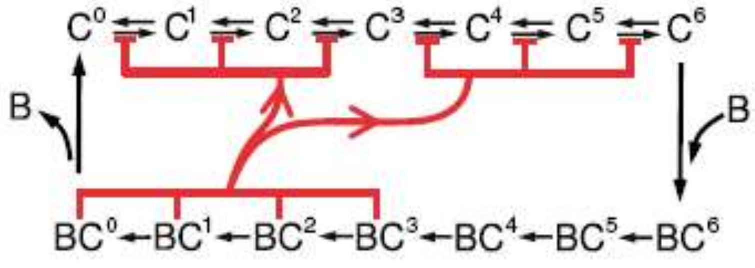
positive feeback (kinase activity of C^6)

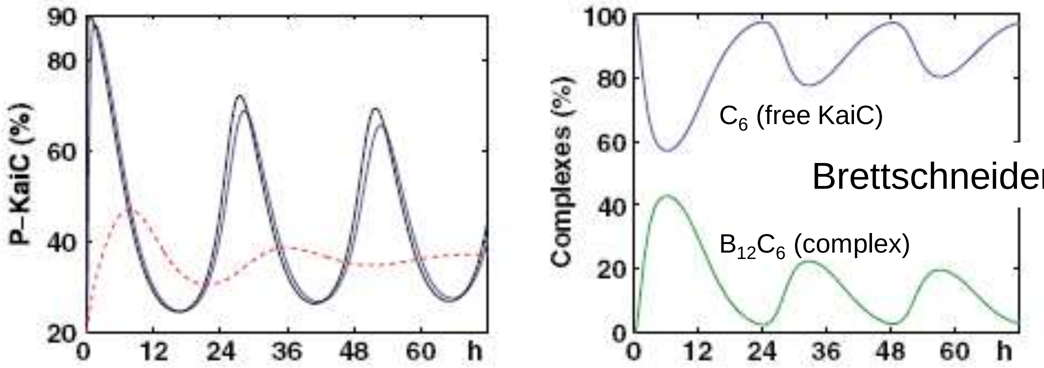




Mehra et al. 2006

negative feedback via KaiA sequestration





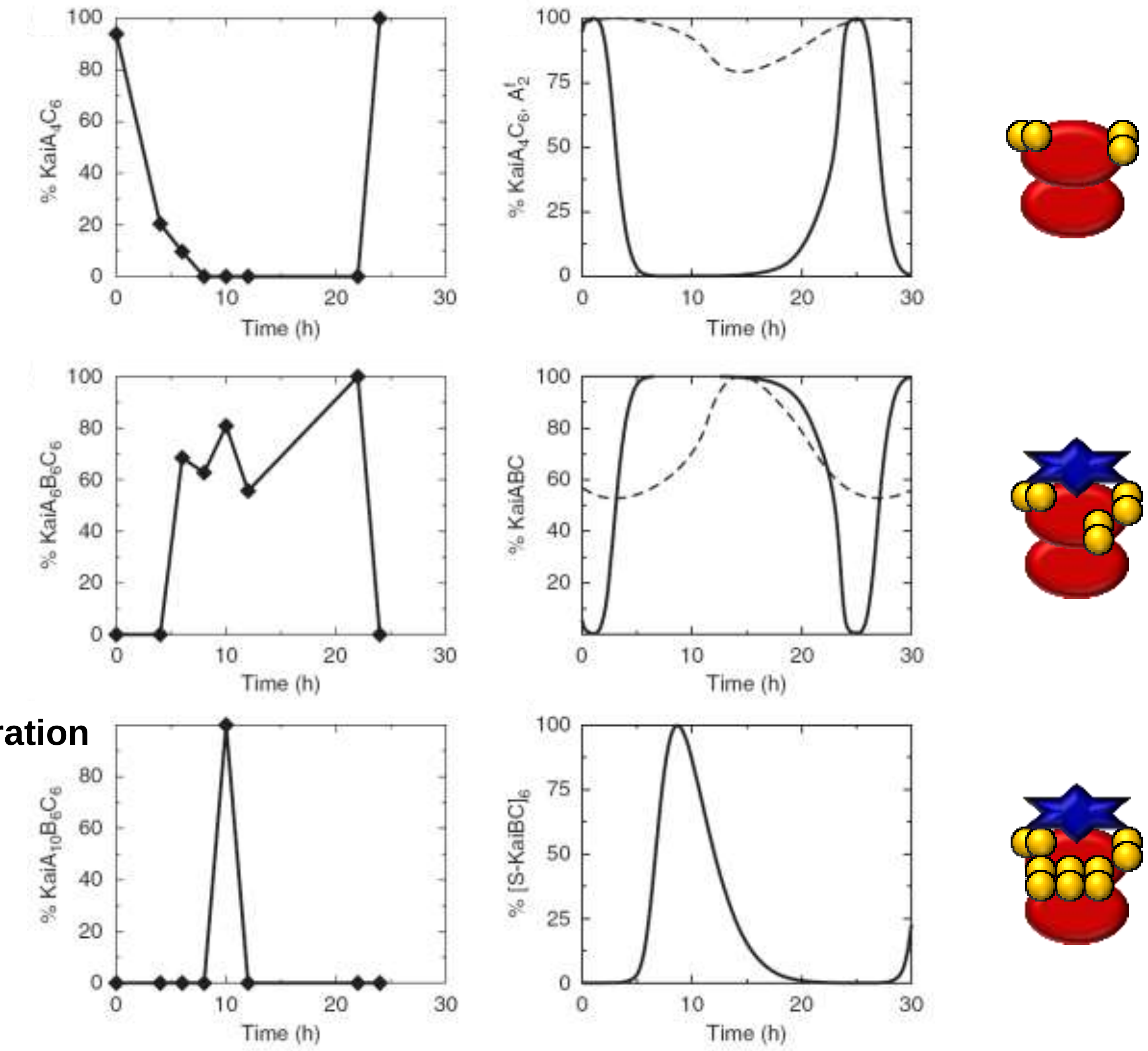
Brettschneider et al. 2010

40-60 % free KaiC

10-15 % KaiC-KaiA

KaiC-KaiB and KaiABC oscillate

… experimental verification by Native Mass Spectrometry



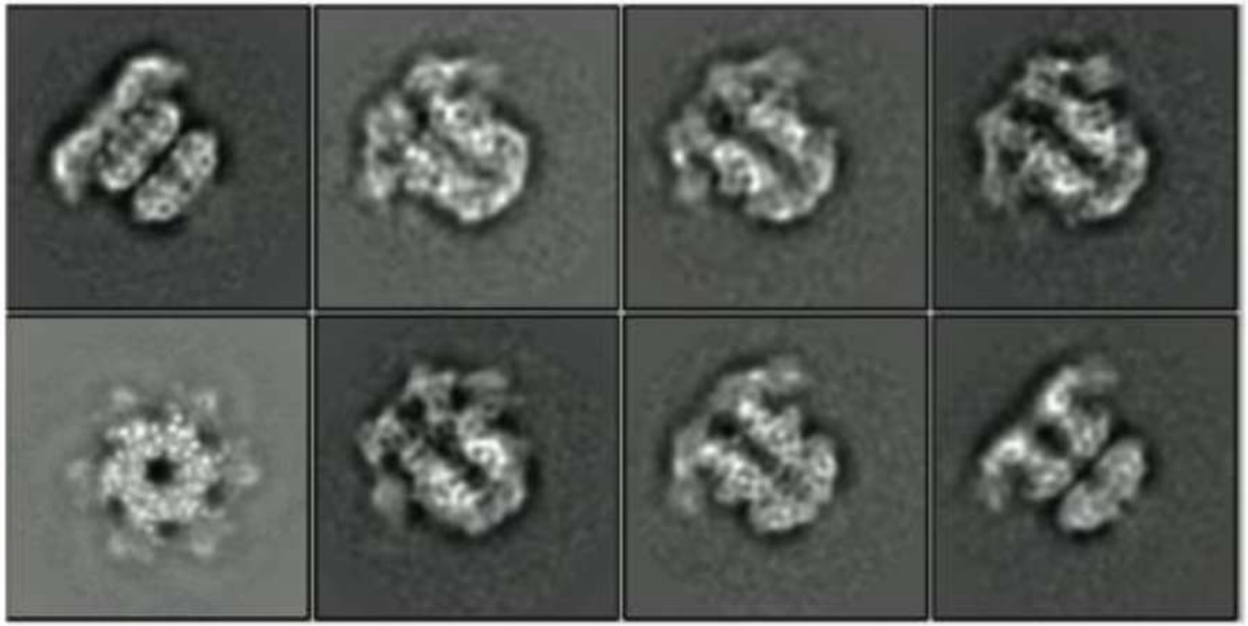
negative feedback via KaiA sequestration

Brettschneider et al. MSB 2010

✓ negative feedback via KaiA sequestration

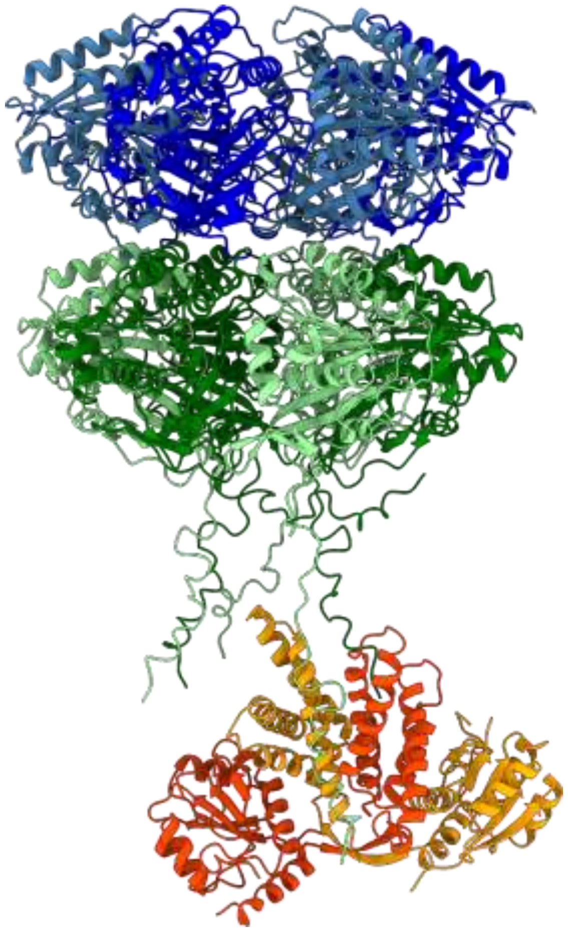
! two different binding sites for KaiA on KaiC:
a site for KaiA sequestration and
a site for catalytic binding

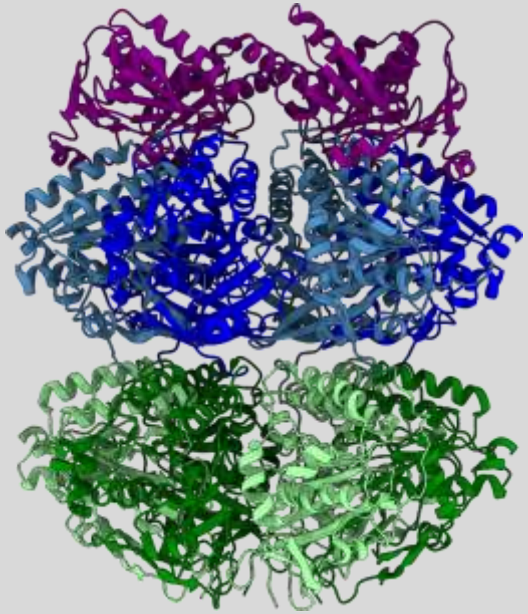
… cryo-EM of KaiCBA allows for 4.7 Å resolution

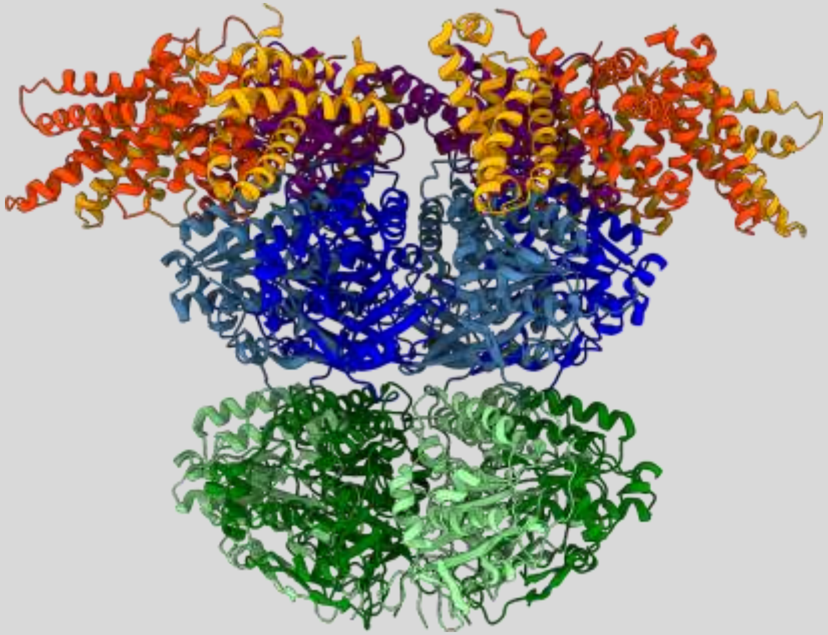


Snijder et al. Science 2017

day





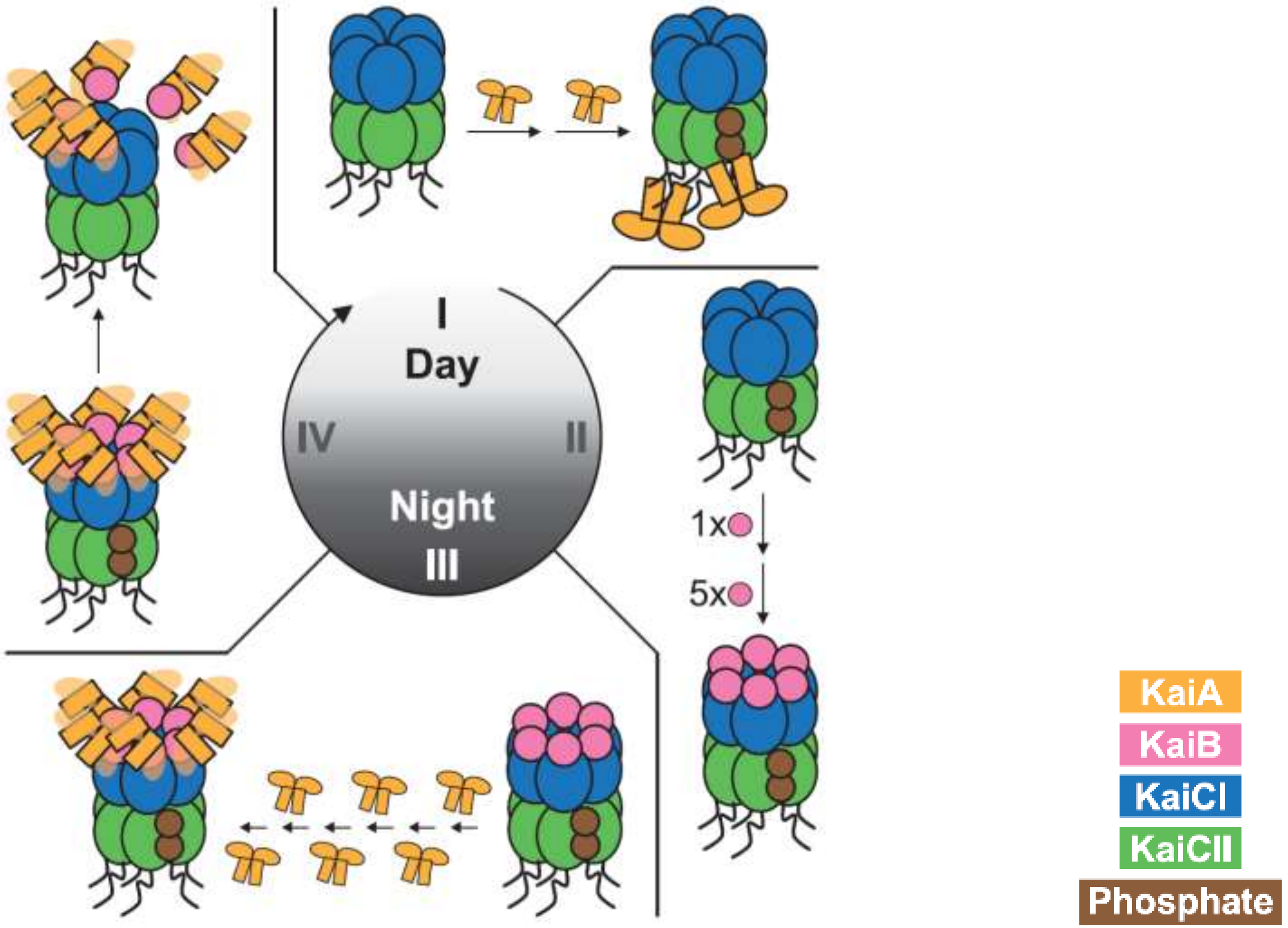


night

Snijder et al. Science 2017

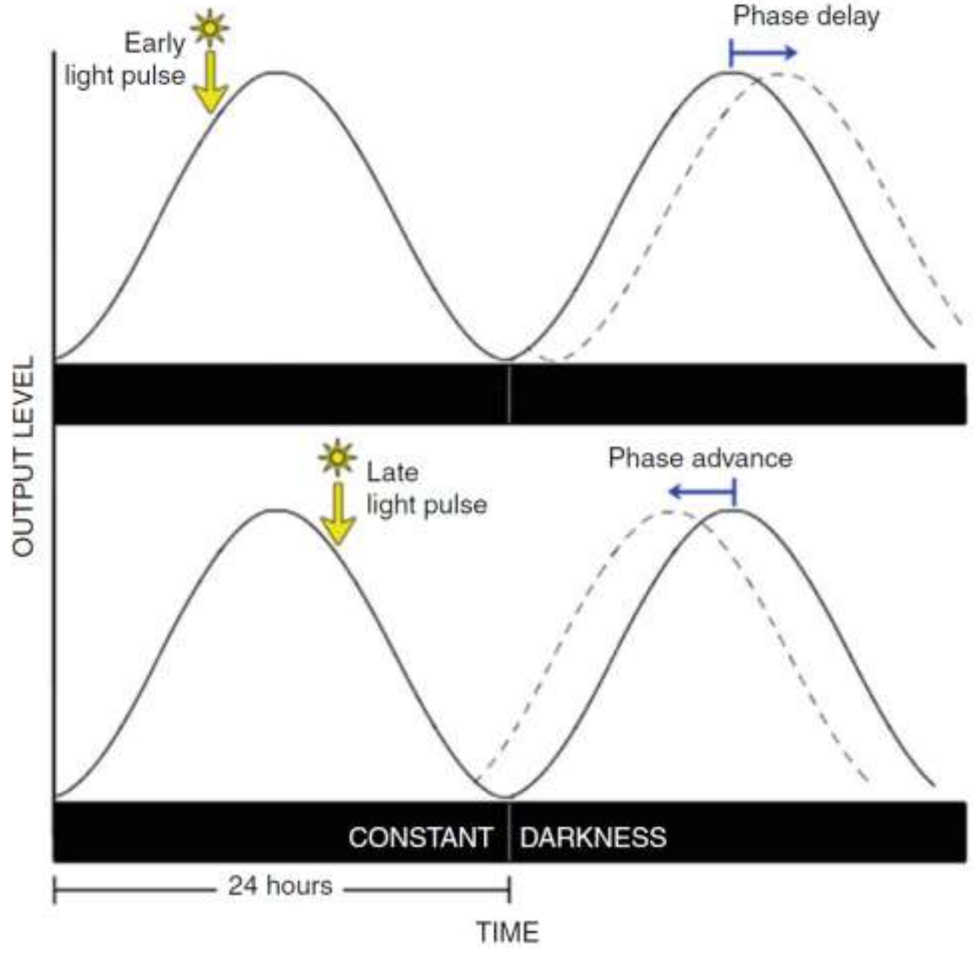
Tseng et al. Science 2017

… 3-protein clock of *Synechococcus elongatus*



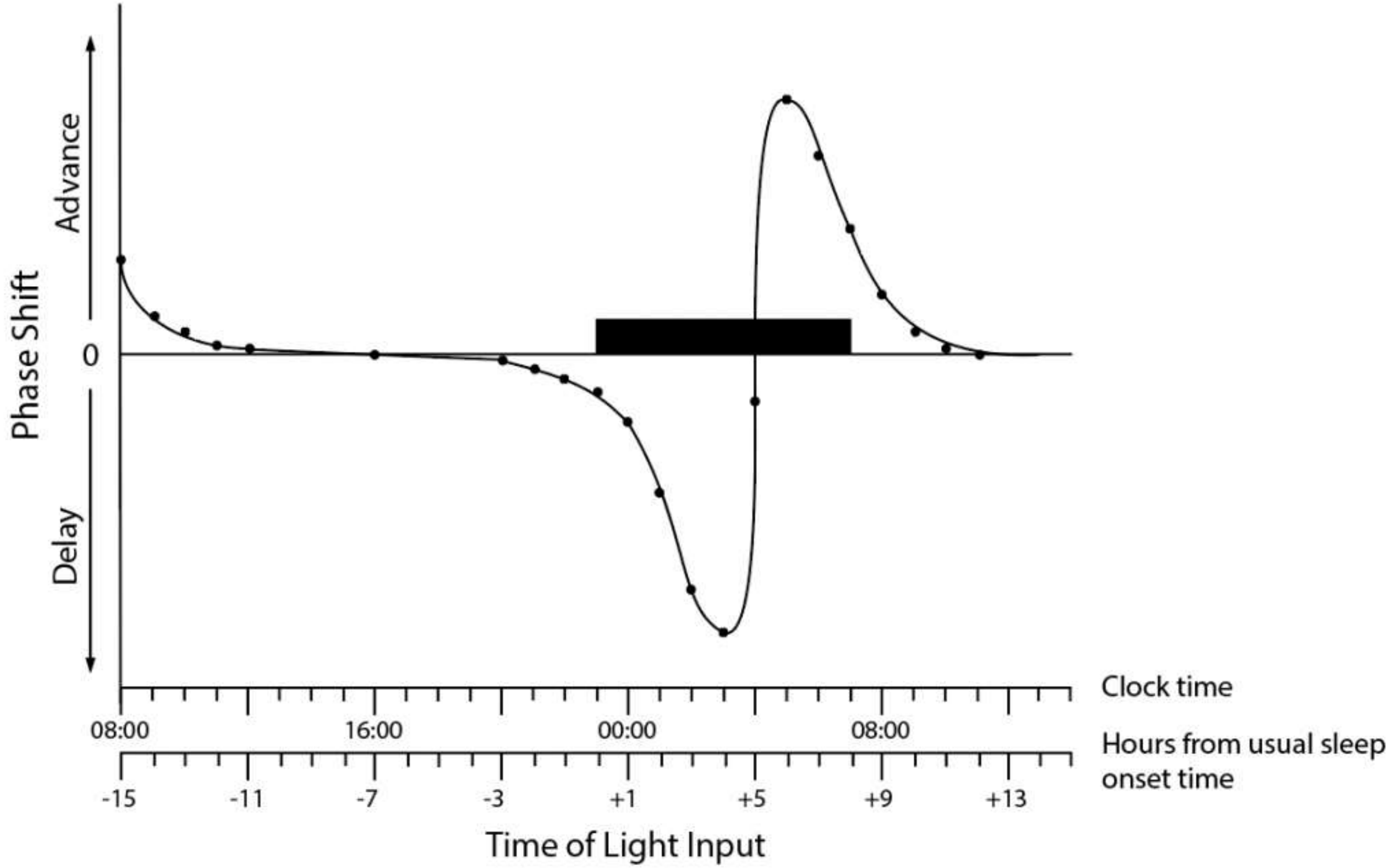
Snijder et al. Science 2017

… phase shifting



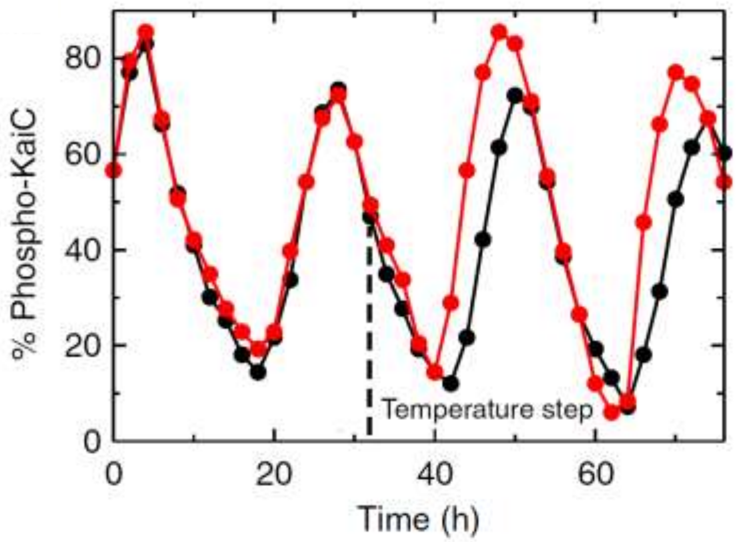
Silver & Rainbow 2013

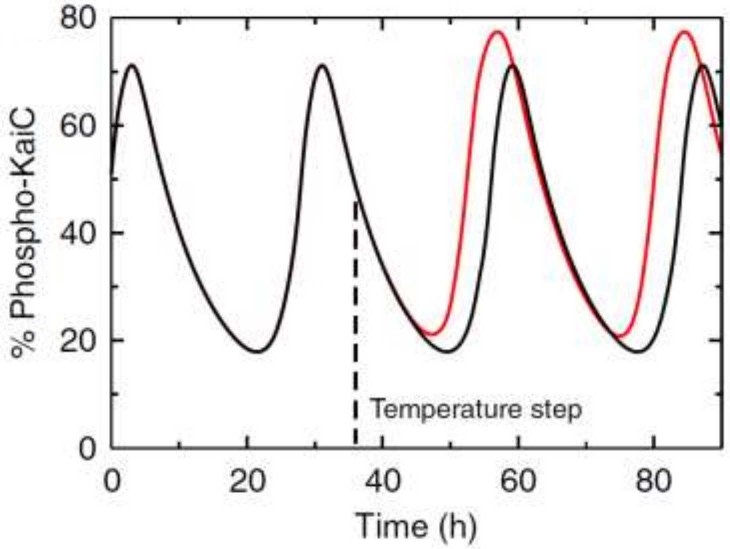
… human phase-response curve

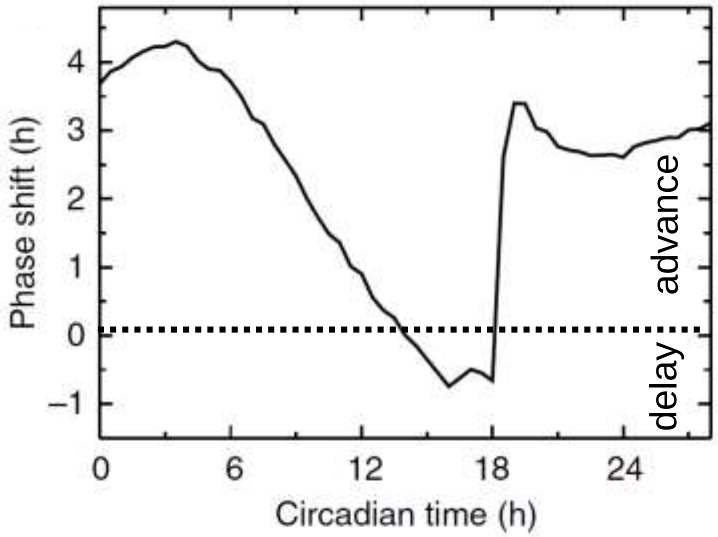


adapted from Burgess et al., 2002

… entrainment of the 3-protein clock by temperature







experimental data

Yoshida et al. 2009

30°C (black line) and 45°C (red line)

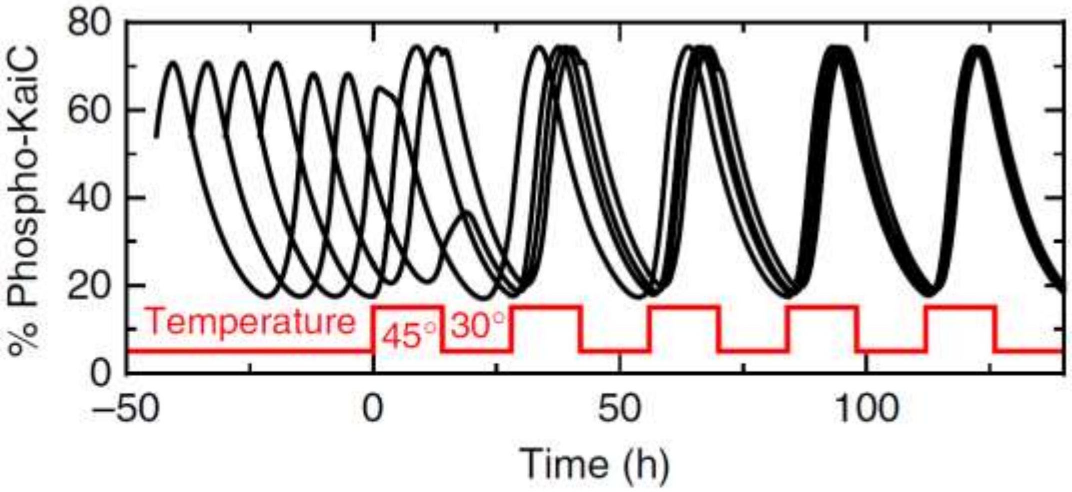
simulation

Brettschneider et al. MSB 2010

phase-response curve

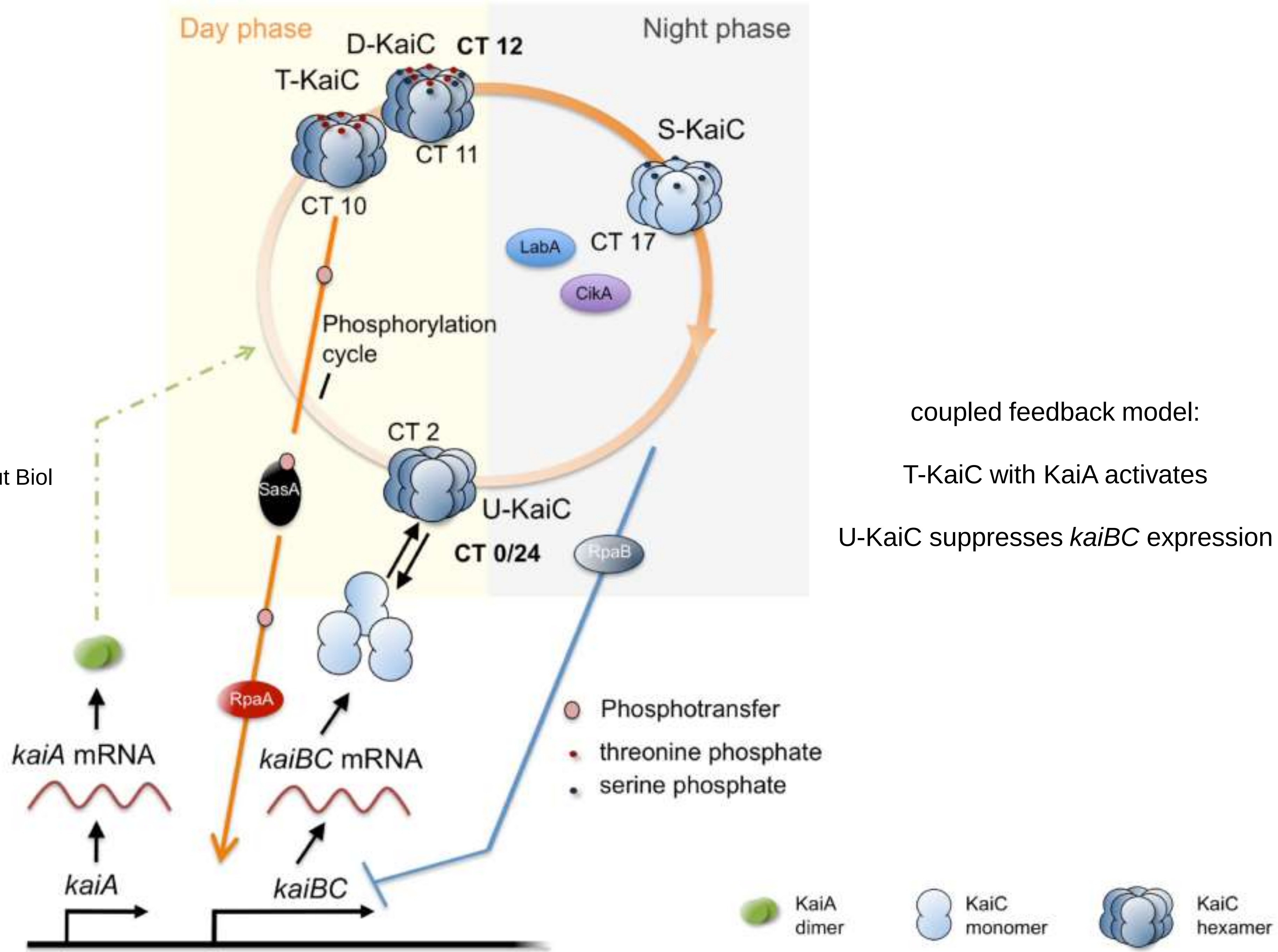
Brettschneider et al. MSB 2010

… predicted phase synchronization dynamics



Brettschneider et al. MSB 2010

Hertel et al. 2013 PLoS Comput Biol

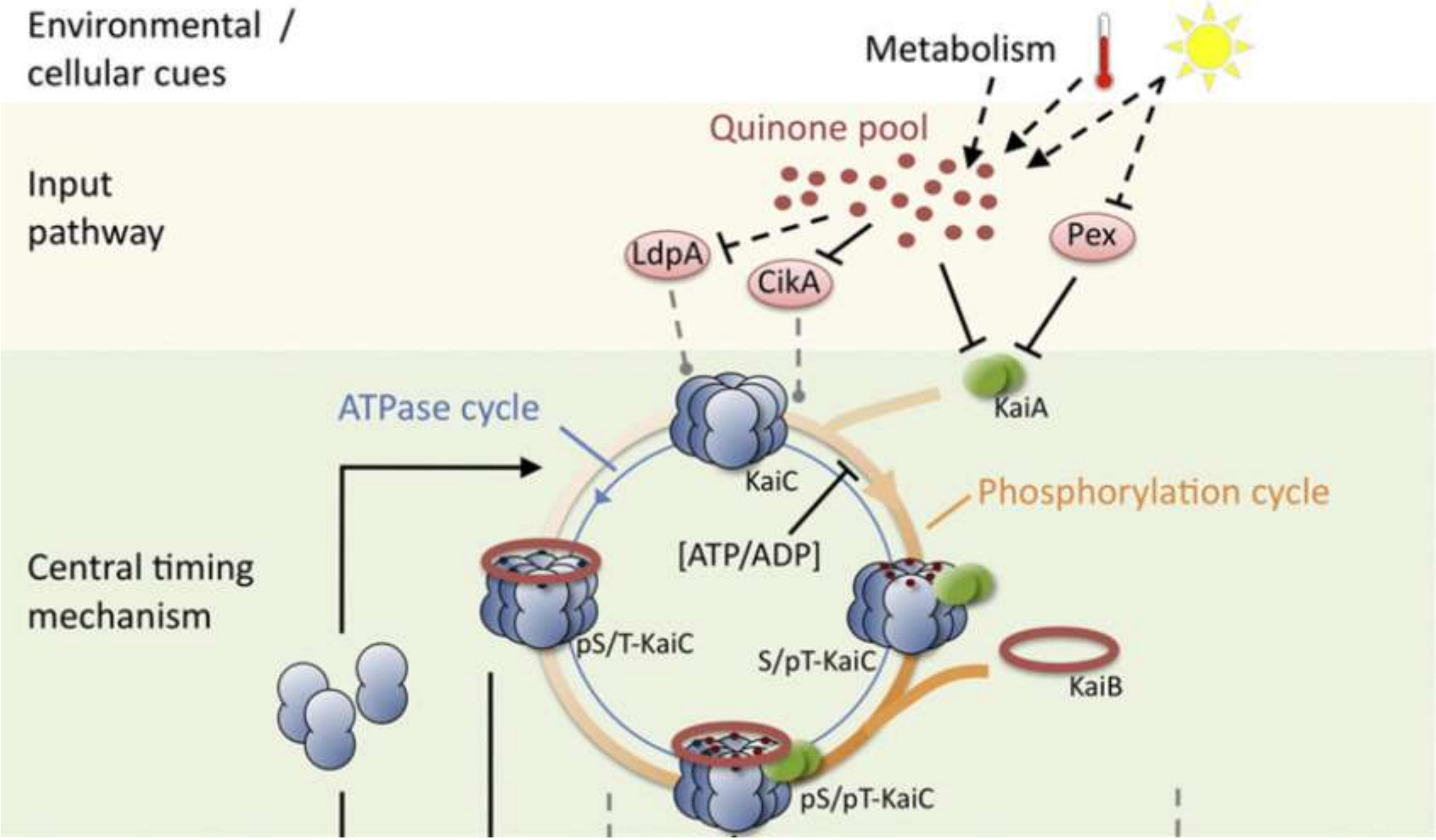


coupled feedback model:

T-KaiC with KaiA activates

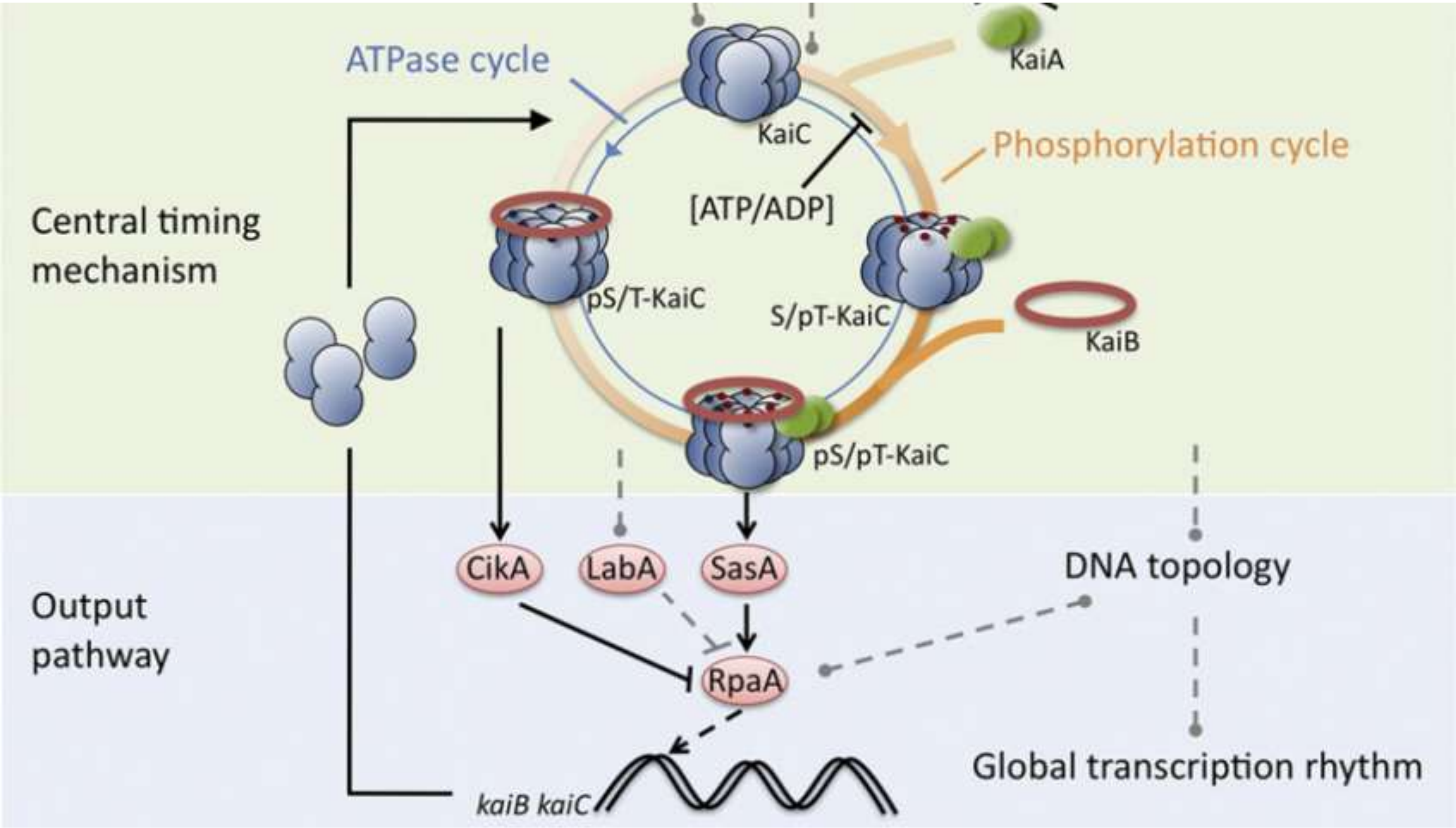
U-KaiC suppresses *kaiBC* expression

… input



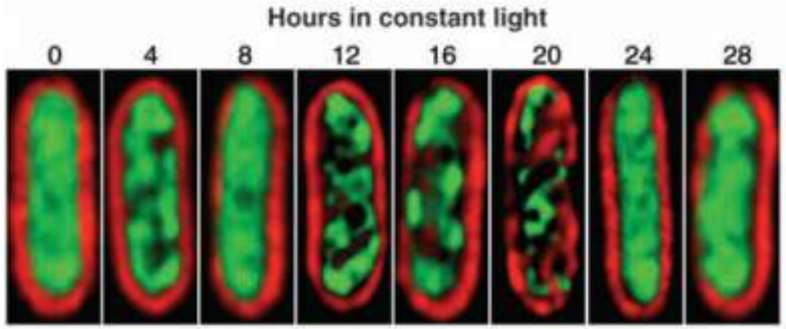
Axmann et al. Marine Genomics 2014 review

… output ?



Axmann et al. Marine Genomics 2014 review

… circadian rhythm of DNA supercoiling



Smith and Williams, 2006; green: DAPI-stained DNA advantages of biological oscillations:

- ✓ temporal organization
- ✓ spatial organisation
- ✓ prediction of repetitive events
- ✓ efficiency
- ✓ precision of control

P. E. Papp Progress in Neurobiology 1986

… open PostDoc position in 2019 !

synthetic micro bio

Heinrich Heine University Düsseldorf