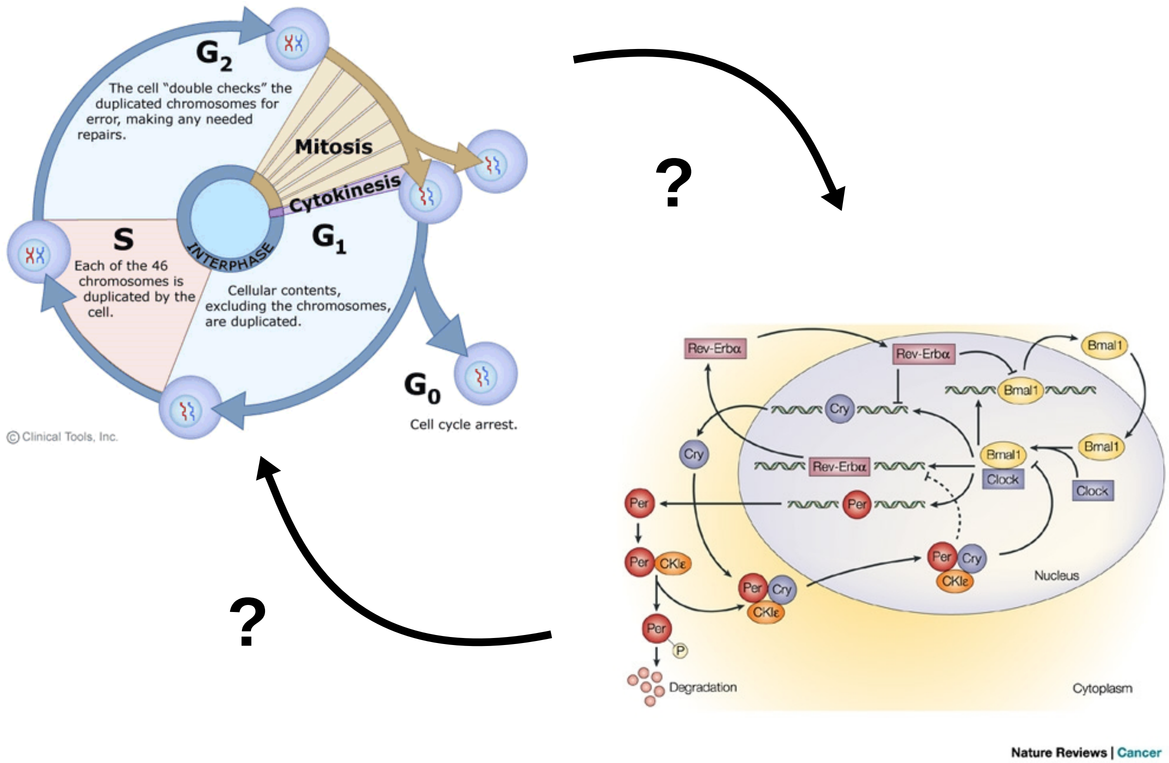
L'horloge circadienne, le cycle cellulaire et leurs interactions

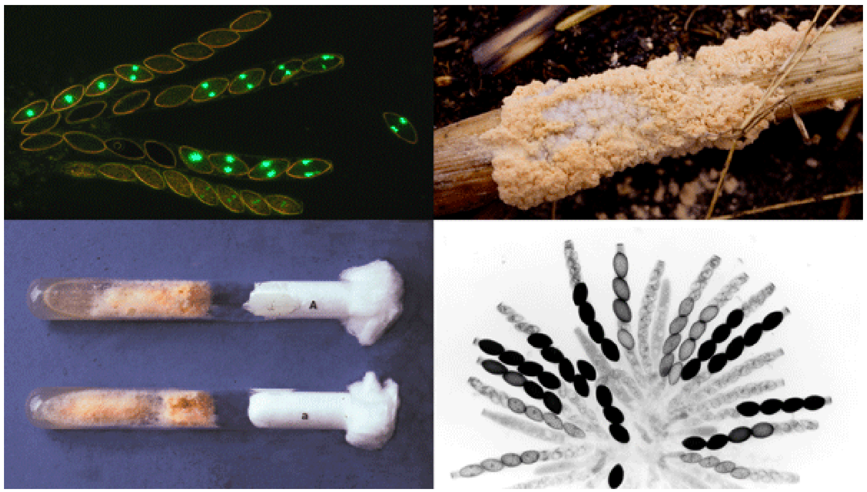


Madalena CHAVES

Inria informatiques mathématiques

Biocore

What do all these living organisms have in common?



Neurospora crassa

Mus musculus

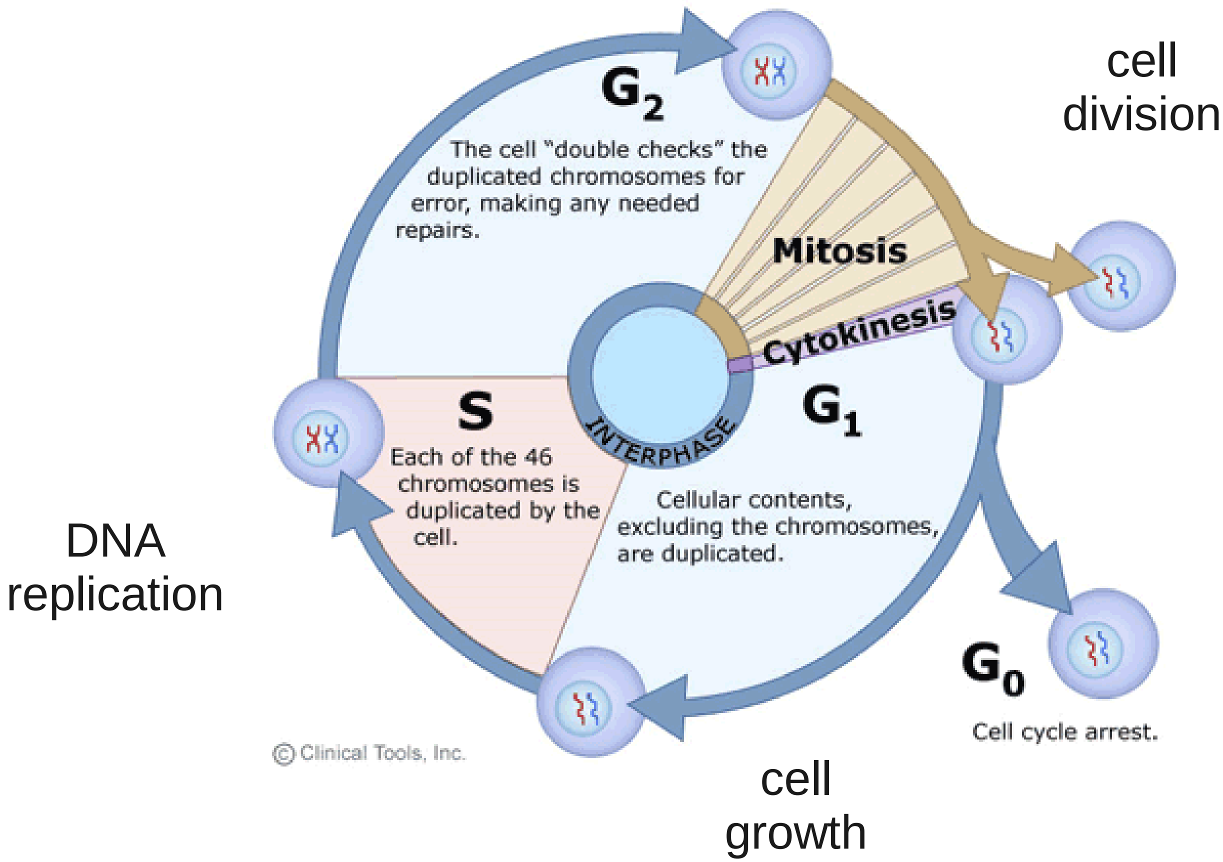
Arabidopsis thaliana

Drosophila melanogaster

Synechococcus elongatus
Cyanobacteria in Baltic sea

Cell cycle

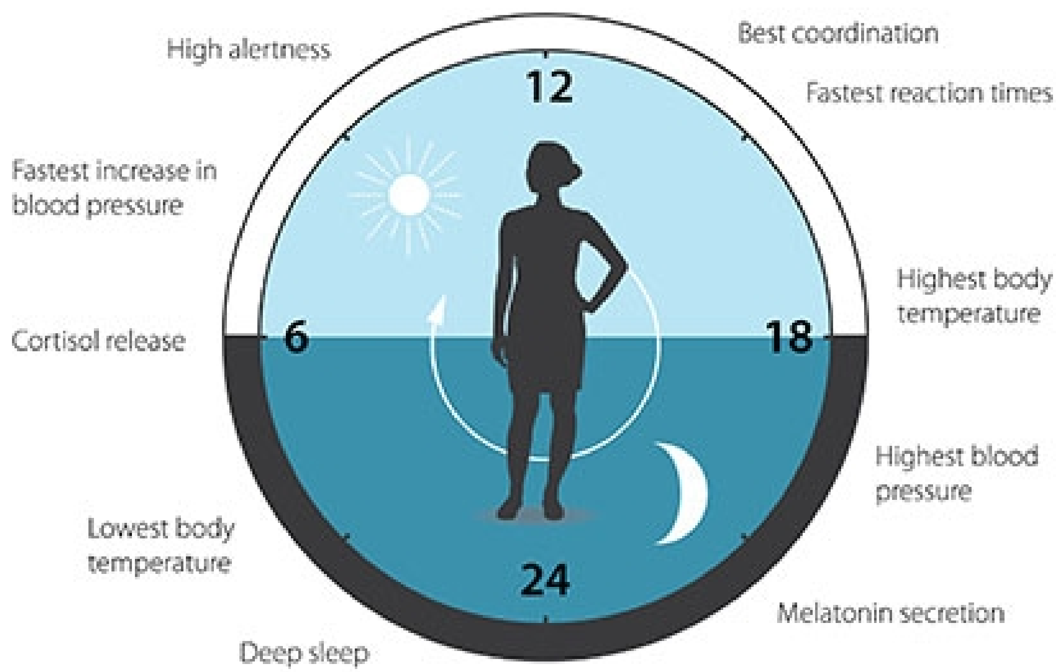
- Cell cycle is the process by which cells growth and divide



Circadian rhythms

- Anticipate environment cycles (day/night) and prepare living organism for upcoming events (eg., expressing required genes)
- Generate an **AUTONOMOUS molecular clock:** cycle goes on independently of other cellular processes
- Temperature compensation: cycle always adjusts to 24h

<u>Cortisol</u>
Lipid degradation,
Glucose production
(energy)



<u>Melatonin</u>
To promote sleep

2017 Nobel Prize

- J. Hall and M. Rosbash (1984) first to identify **gene “period”,** responsible for circadian rhythm in drosophila
- Hypothesis: protein PER contributes to **eventually repress its own gene**, thus creating a negative feedback loop

Drosophila Melanogaster
→ mouche du vinaigre

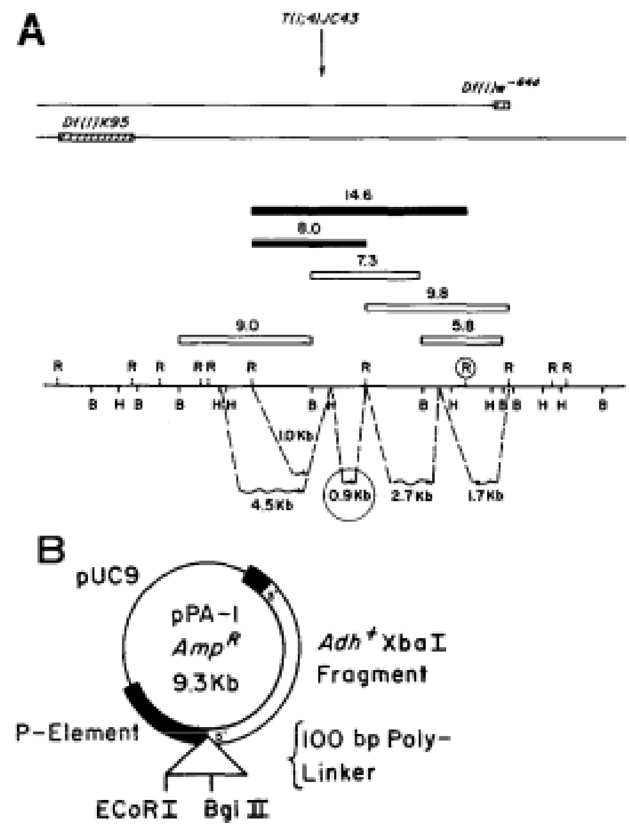
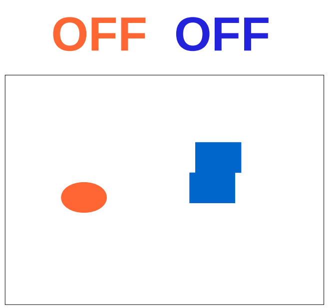
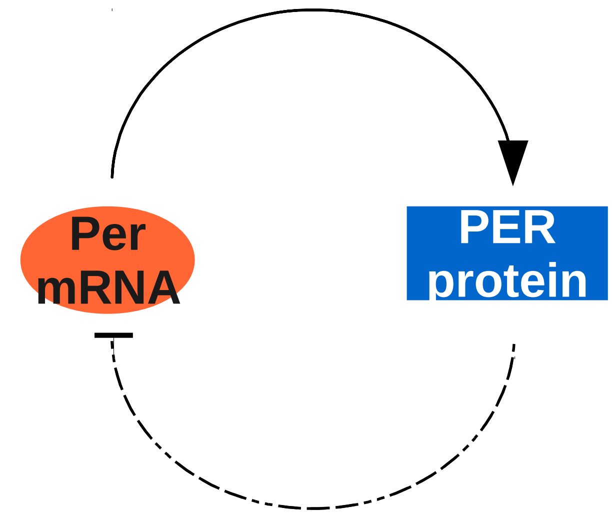


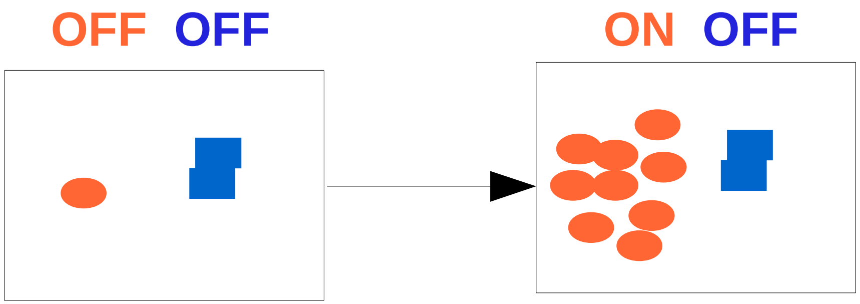
Figure 1. Molecular Map of Subclones in the *per* Region Used in Transfor-
mation, with Reference to Chromosomal Breakpoints and Transcripts from

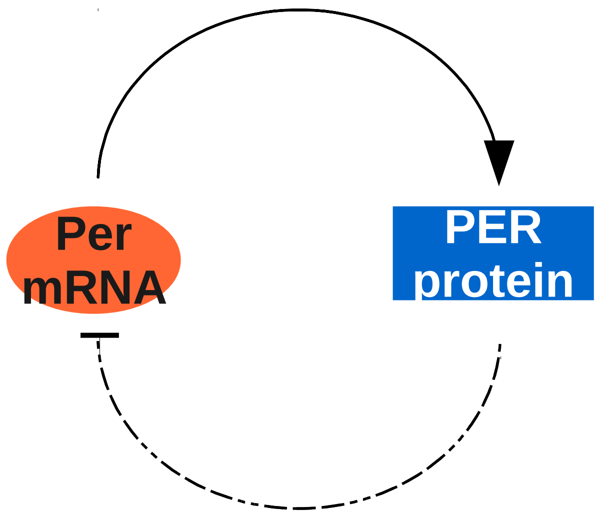
Negative feedback loop



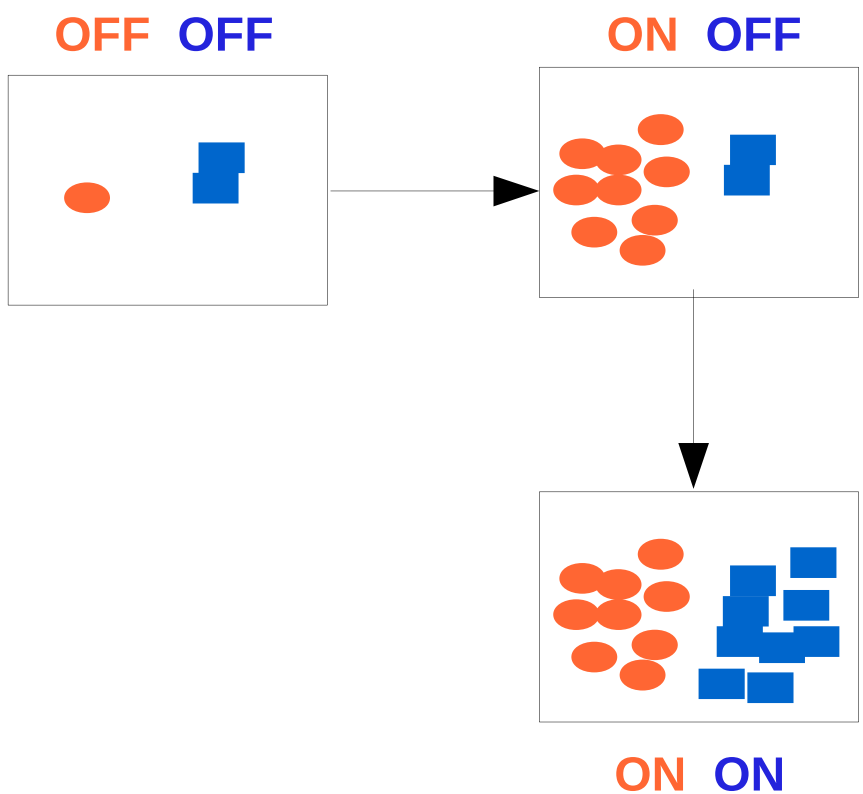


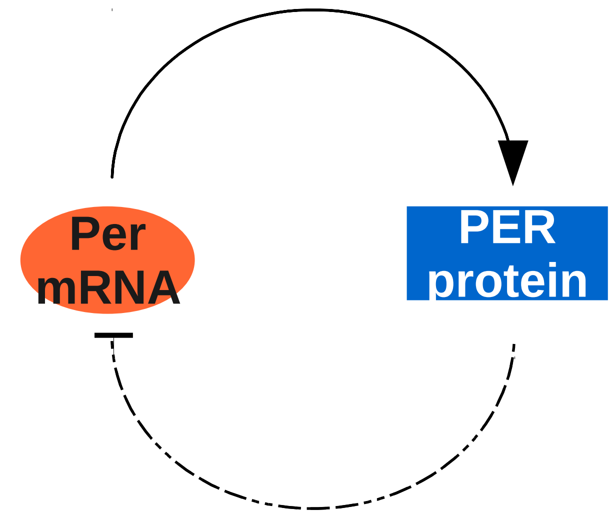
Negative feedback loop



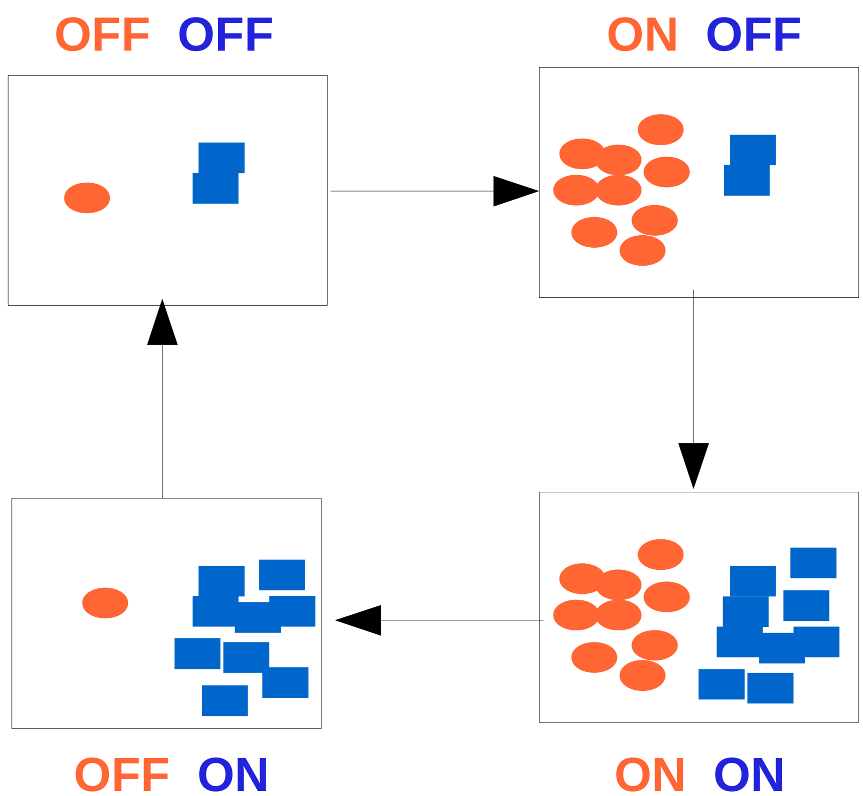


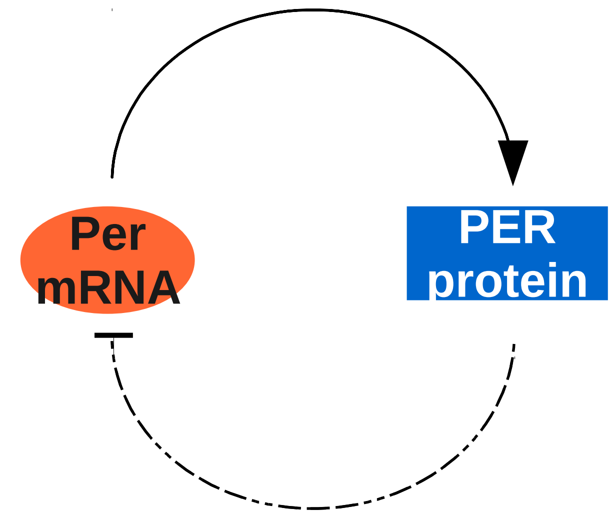
Negative feedback loop





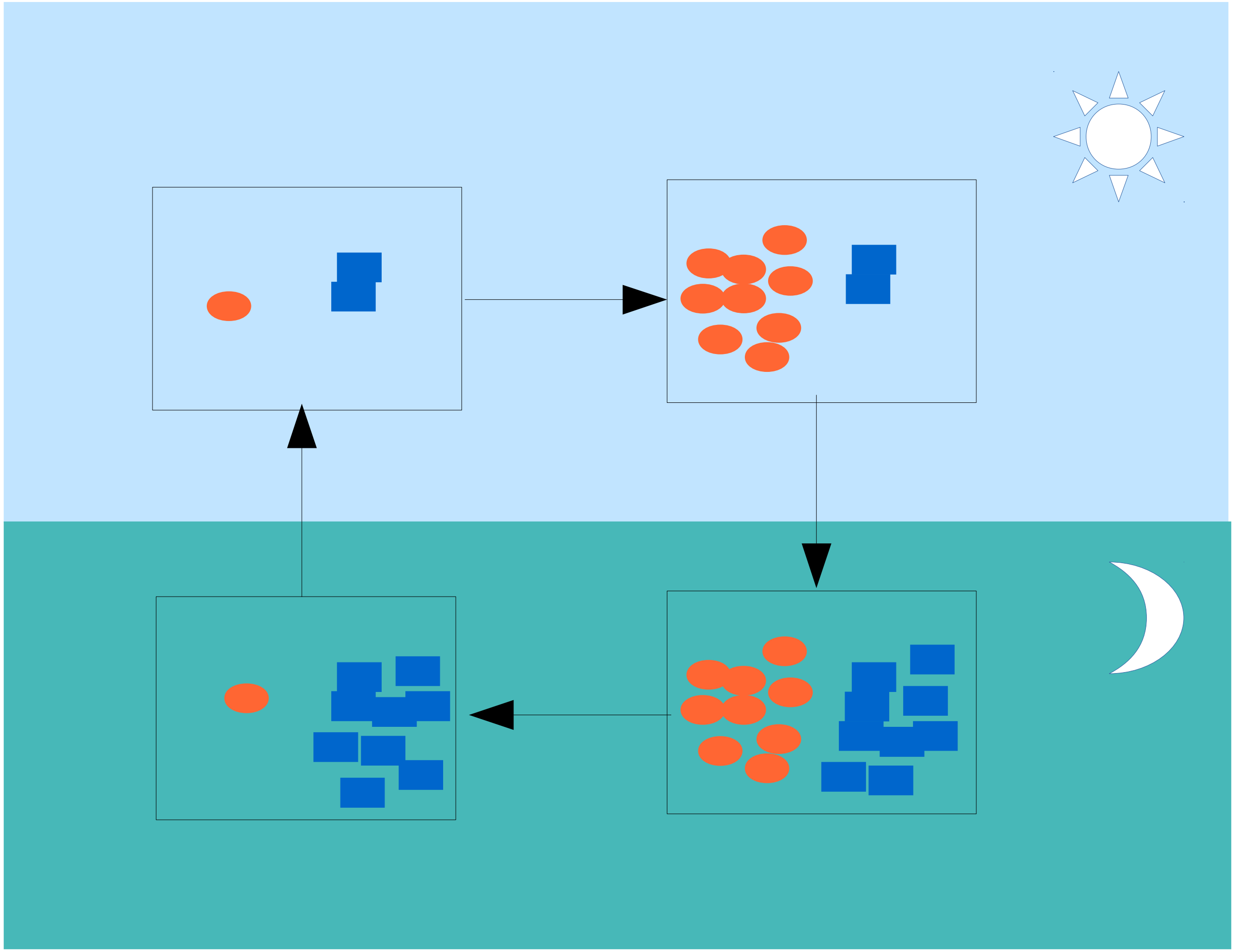
Negative feedback loop

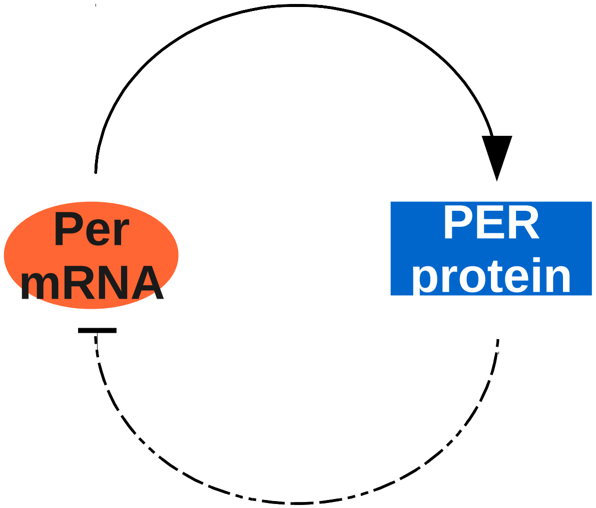




Generates an **oscillatory Cycle** !

Negative feedback loop





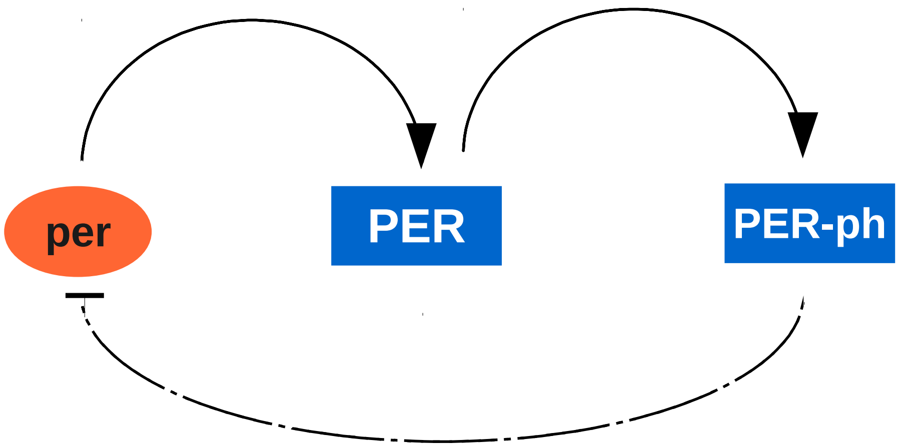
PER protein accumulates during the night

Zehring,..., Hall and Rosbash, Cell 1984

A basic negative feedback loop is not enough

- How to guarantee that oscillations are sustained ?

→ increase the number of steps:

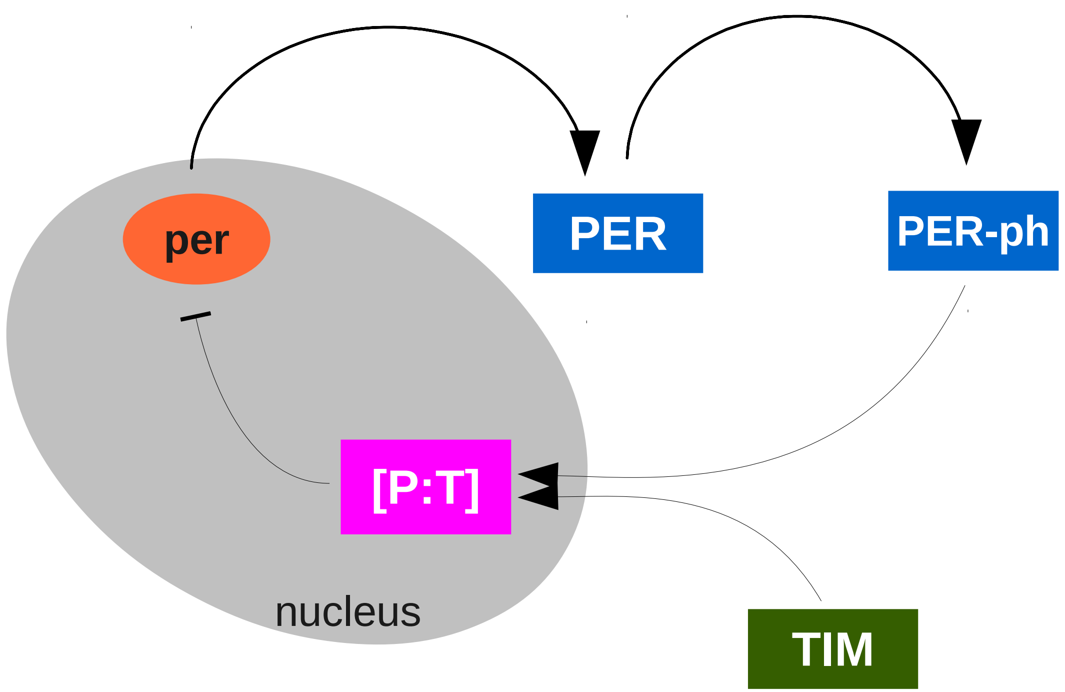


A basic negative feedback loop is not enough

- How to guarantee that oscillations are sustained ?

  → increase the number of steps:

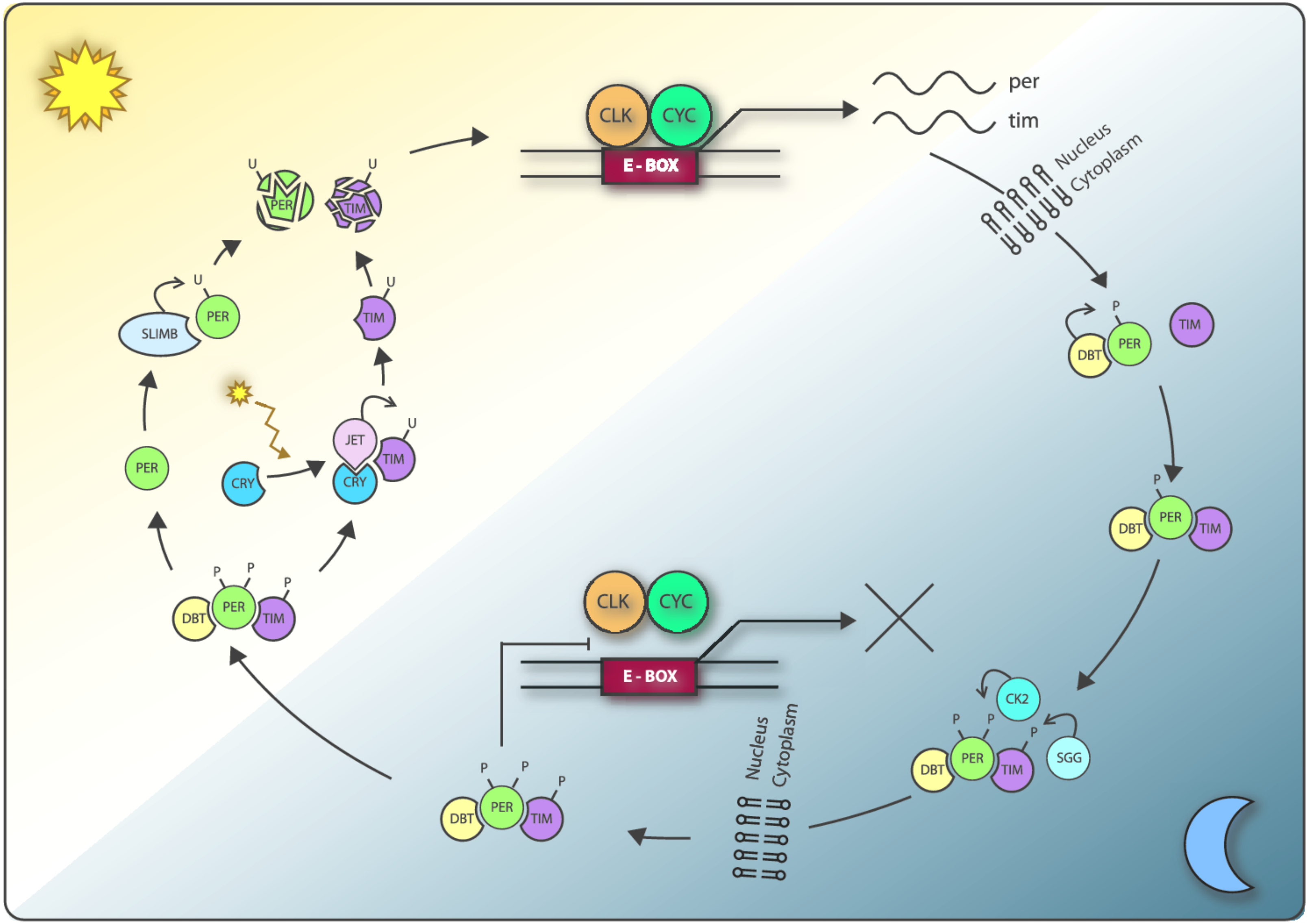
- How to generate a **24 hour cycle** ?



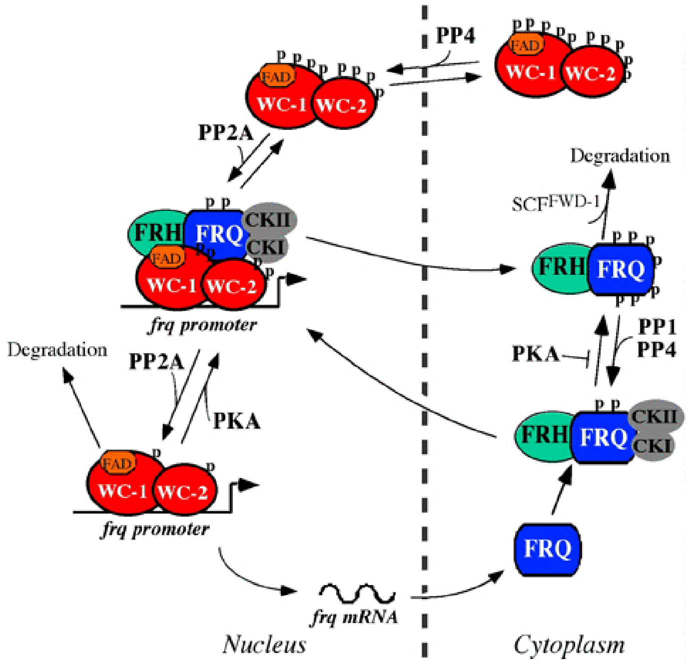
Other proteins are involved that impose "waiting" times

"timeless" (TIM) and "doubletime", discovered by M. Young 1994, 98

Circadian clock in Drosophila

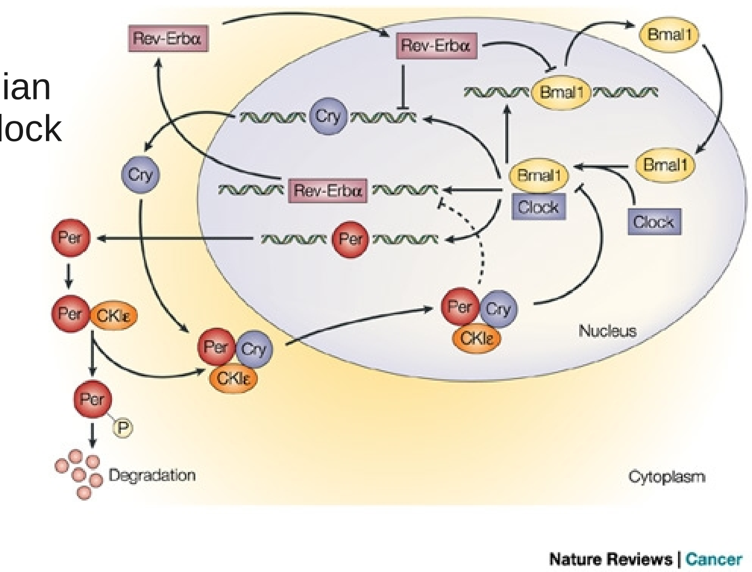


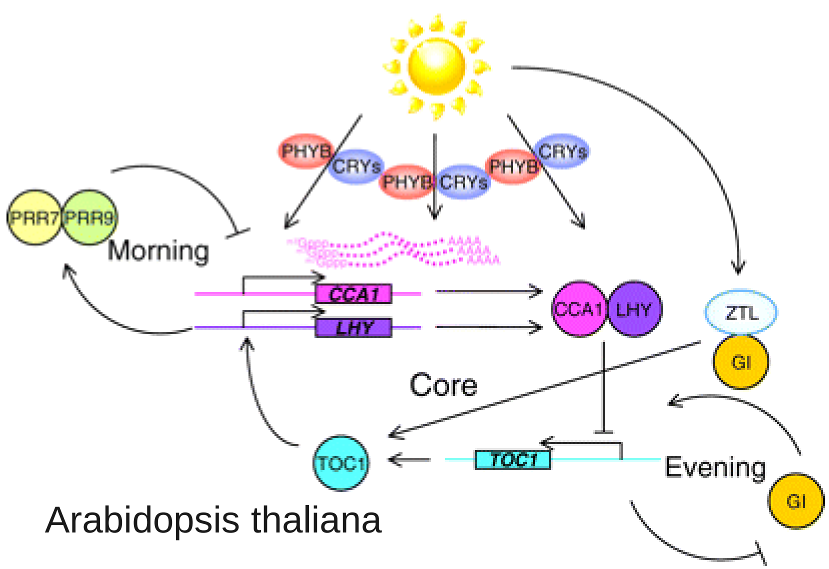
Similar clock mechanisms across organisms



Neurospora crassa

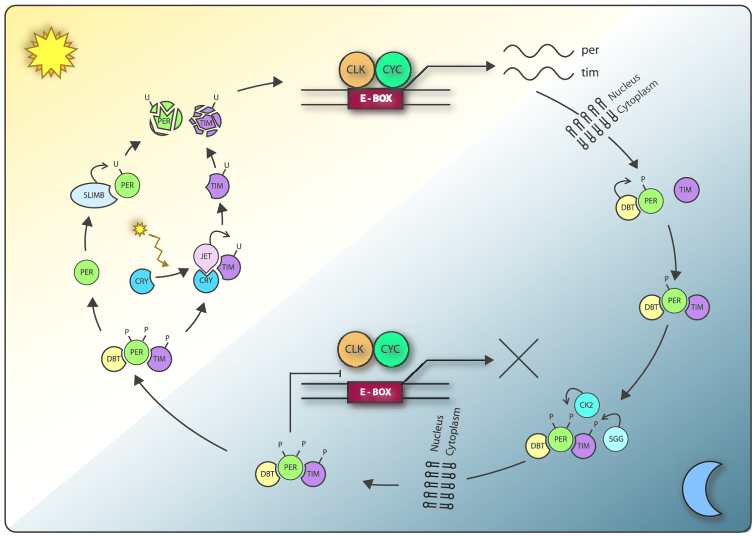
Mammalian clock



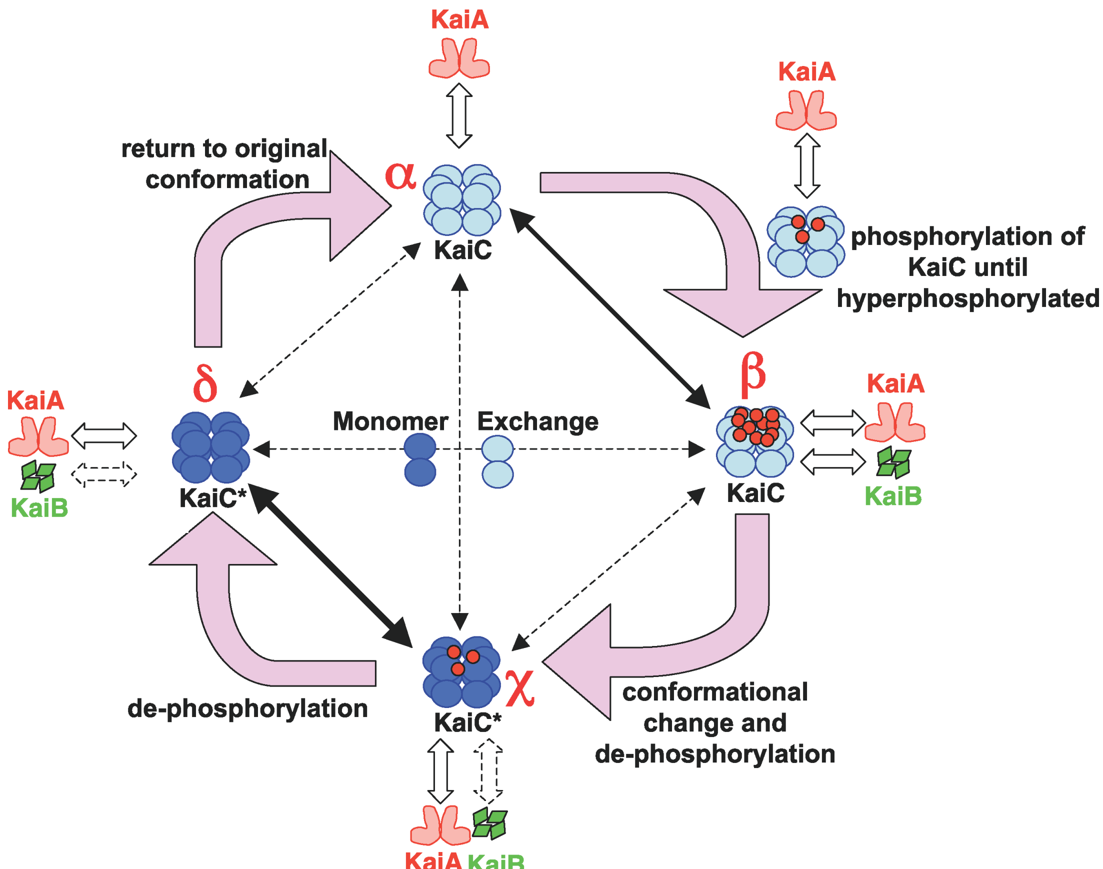


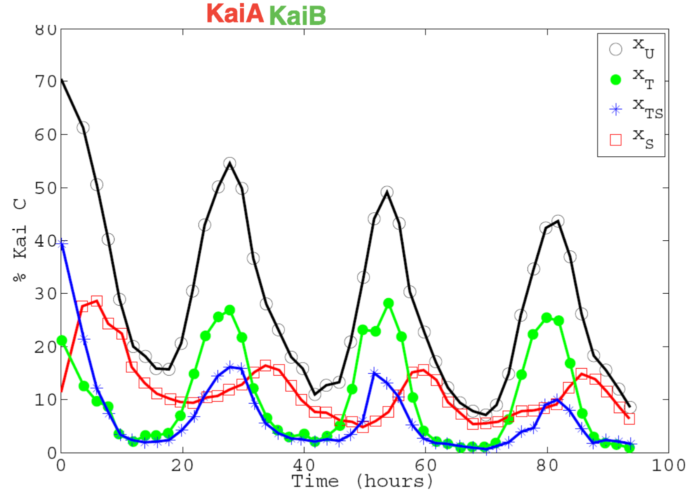
Arabidopsis thaliana

Drosophila melanogaster



Autonomous clock: cyanobacteria



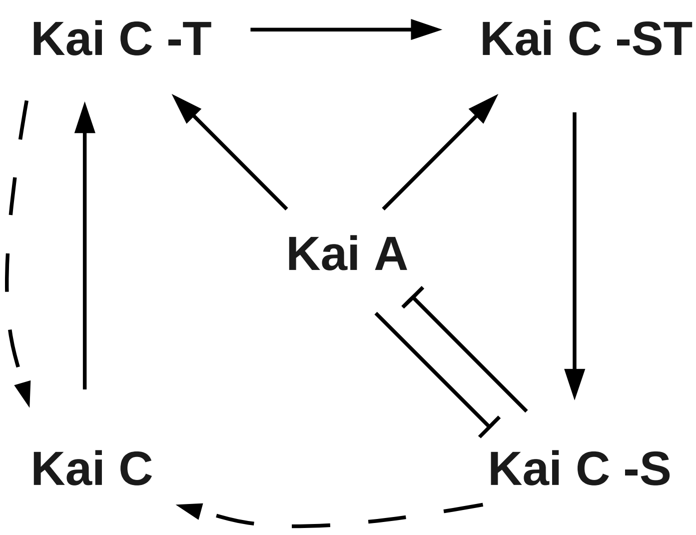


“In vitro” system:
only proteins in a test tube;
no genes, no transcription
or translation involved !

Oscillations are due to an ordered cycle of phosphorylations and dephosphorylations of a protein, Kai C

Kondo lab, 2005-2007,
Rust et al. 2007

Modeling cyanobacteria clock



$$\dot{x}_A = k_A h^-(x_S, \theta_S) - \gamma_A x_A$$
$$\dot{x}_T = k_T h^+(x_U, \theta_U) h^+(x_A, \theta_A) - \gamma_T x_T$$
$$\dot{x}_{TS} = k_{TS} h^+(x_T, \theta_T) h^+(x_A, \theta_A) - \gamma_{TS} x_{TS}$$
$$\dot{x}_S = k_S h^+(x_{TS}, \theta_{TS}) h^-(x_A, \theta_{A2}) - \gamma_S x_S$$

$$x_U = x_{total} - x_T - x_{TS} - x_S$$

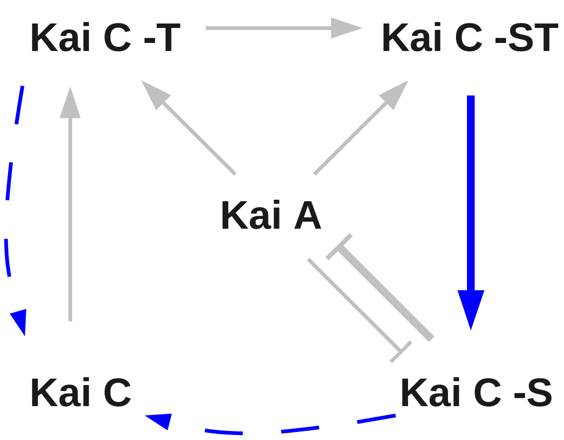
Need to **calibrate model** that is find the values of parameters:

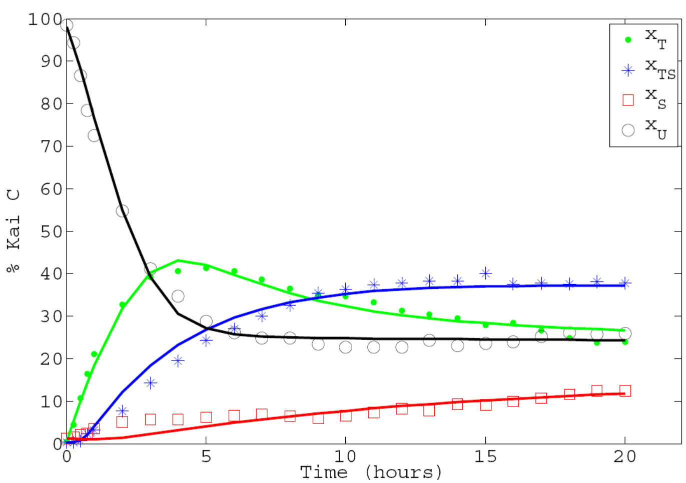
$$k_A, k_T, k_{TS}, k_S, \gamma_A, \gamma_T, \gamma_{TS}, \gamma_S, \ldots$$

Chaves & Preto, Chaos 2013

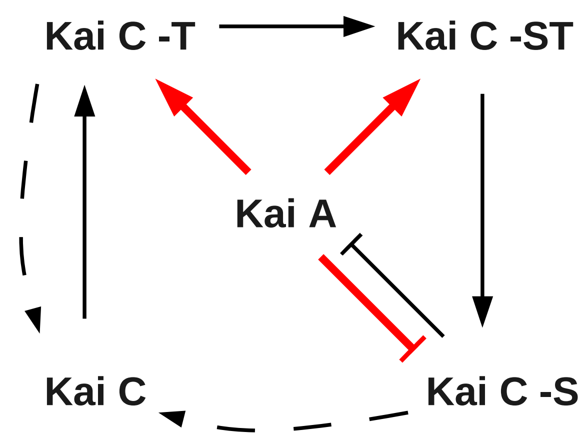
Model calibration

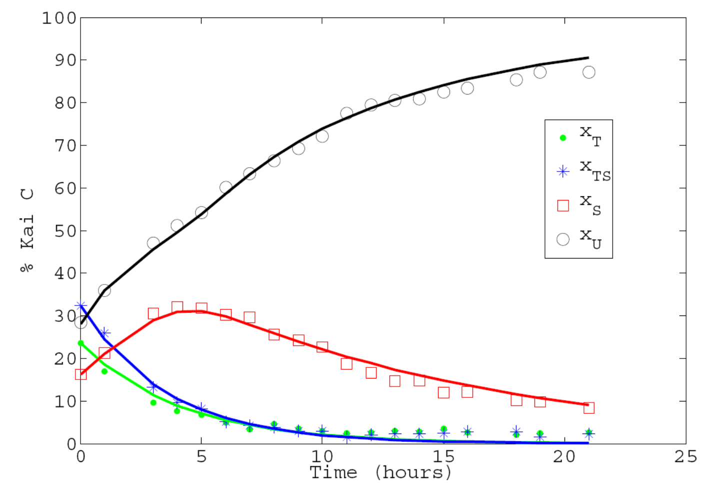
Experiment 1
Dephosphorylation





Experiment 2
Phosphorylation



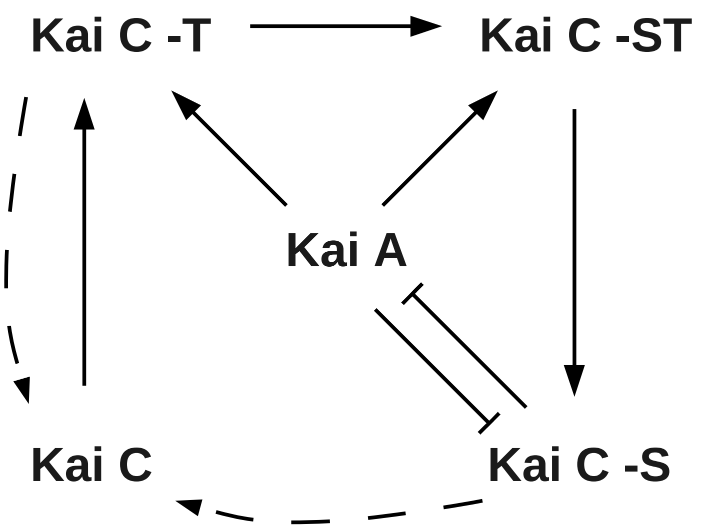


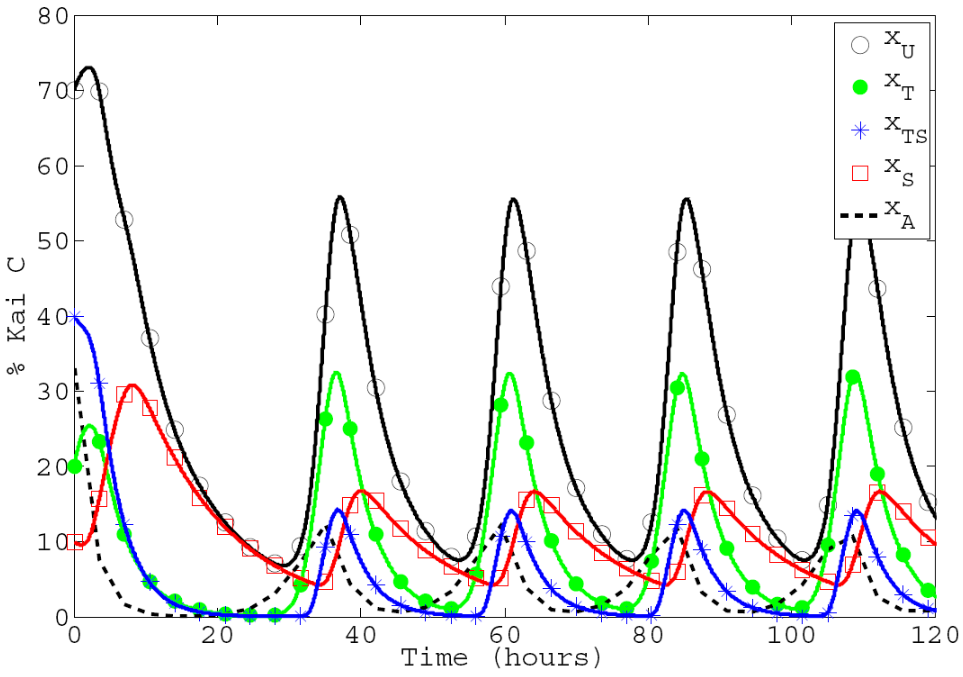
Data sets: Rust et al, Science 2007

Model validation

Values of parameters were obtained from **two distinct sets of experiments, each for a “partial” model.**

Putting all parameters together yields the correct oscillatory solution!

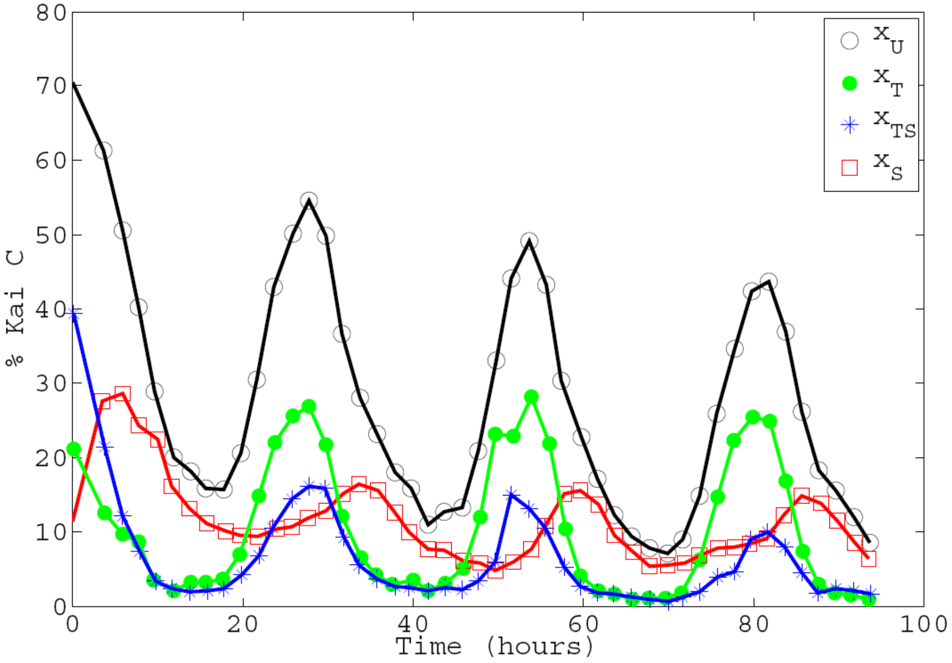


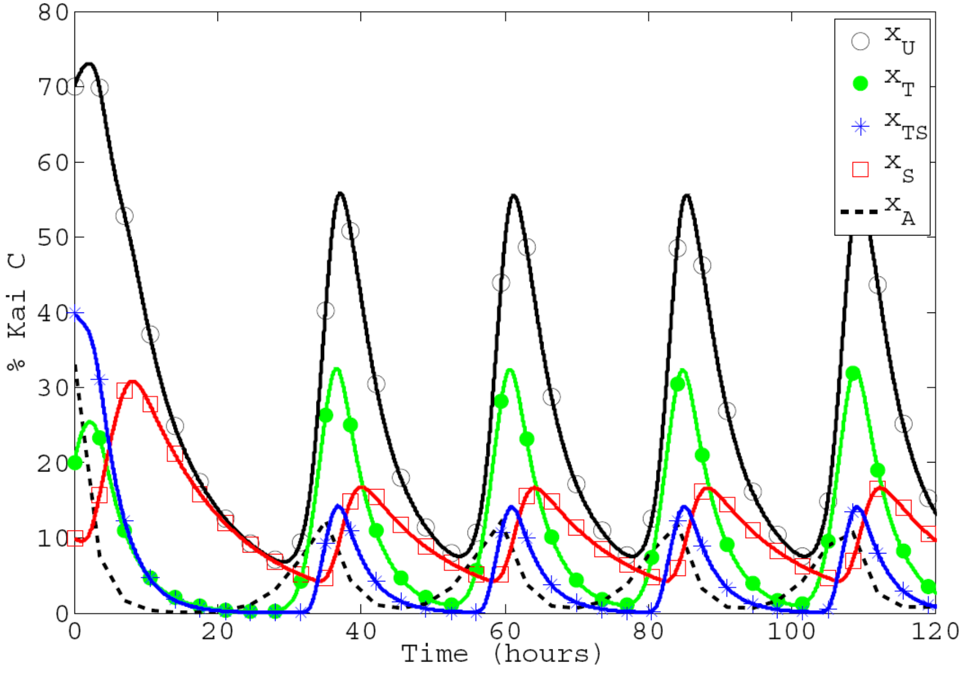


Data vs. model comparison

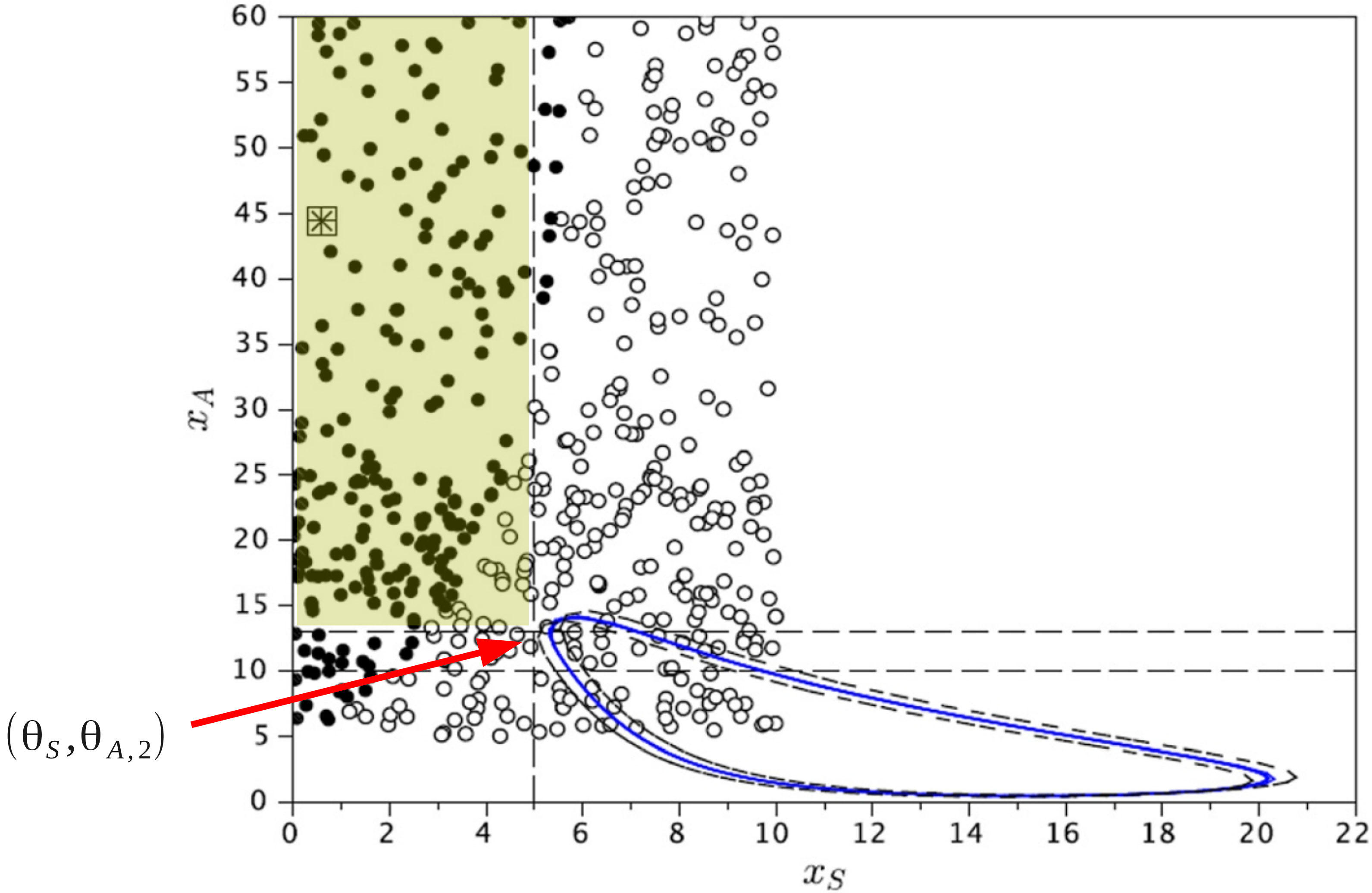
Values of parameters were obtained from **two distinct sets of experiments, each for a “partial” model.**

Putting all parameters together yields the correct oscillatory solution!





Model analysis



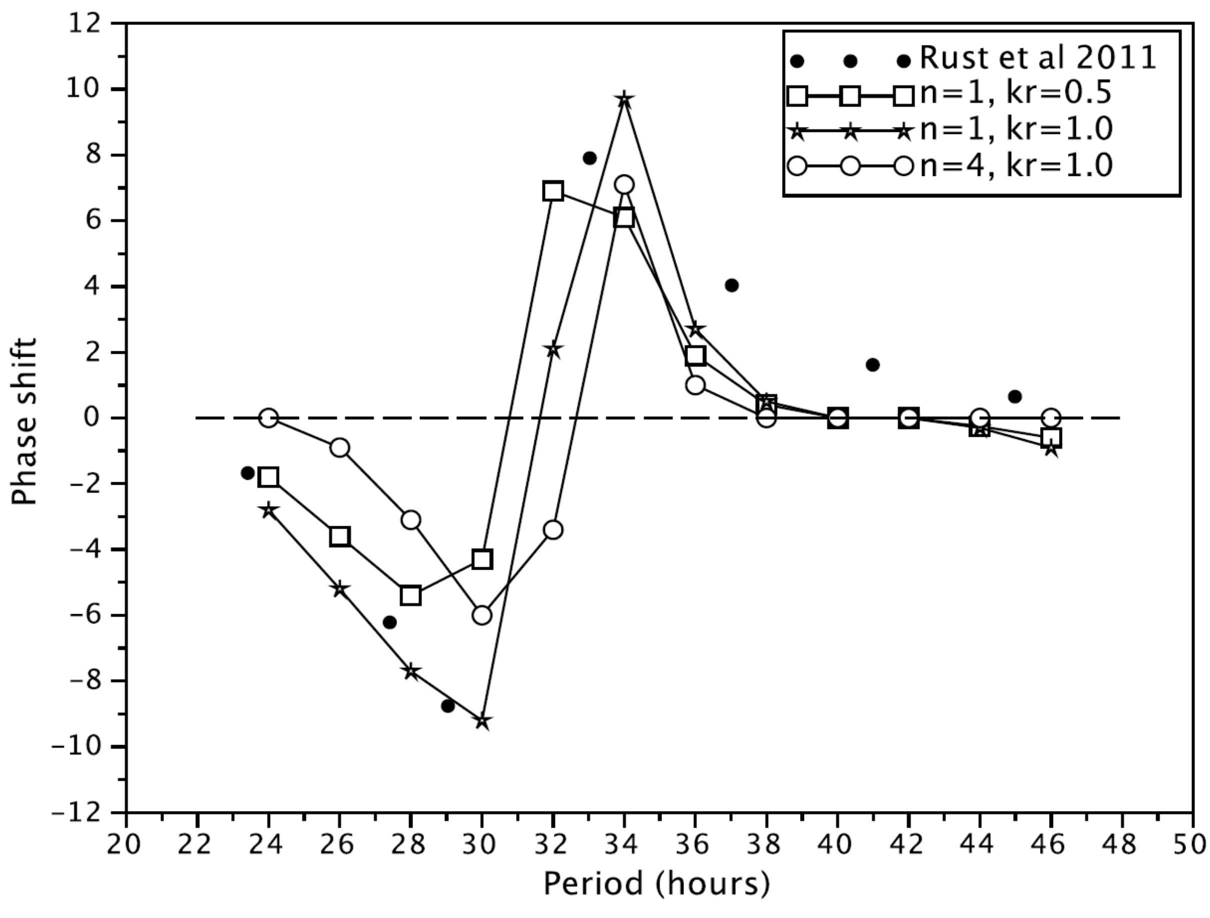
Shaded: Invariant Region (PWA)

Closed circles: Basin of attraction of x*

Open circles: Basin of attraction of periodic orbit

PREDICTION: there exist critical points in the periodic orbit, where the system my be perturbed and the **circadian cycle arrested** – such as when adding a large amount of Kai A.

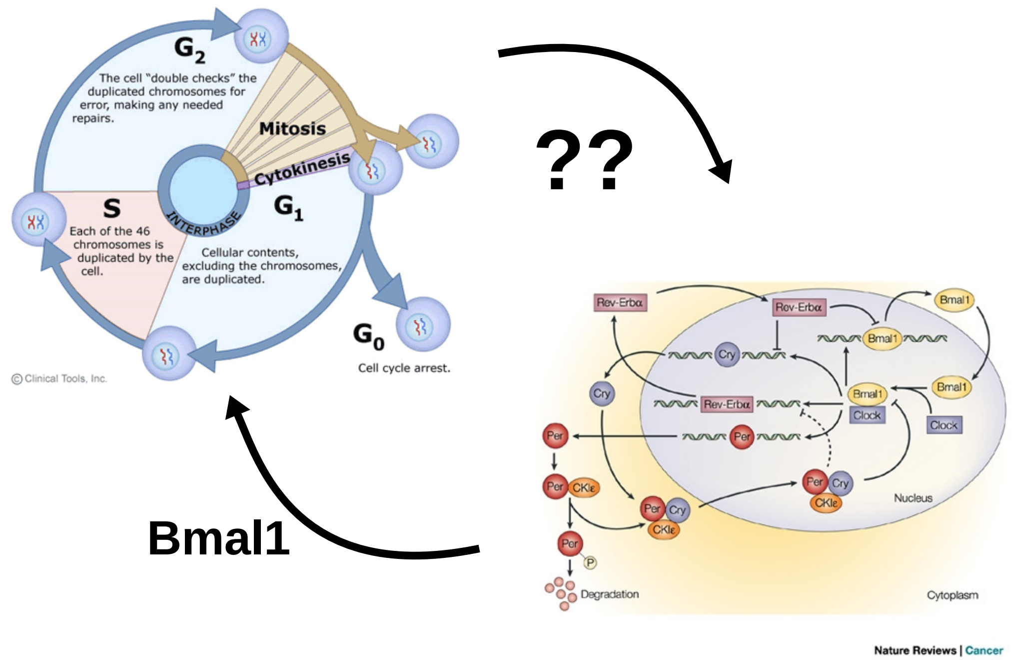
Model predictions: response to light



Chaves & Preto, Chaos 2013

Phase response to 5h pulses of ADP, applied at different instants during the circadian cycle. This experiment mimics clock entrainment by light – which varies the ratio ATP/(ATP+ADP).

Cell cycle and circadian rhythms
How do they interact?



- The general belief (cf. cyanobacteria): **autonomous molecular clock**
- **Very few** possible links are known from clock to cell cycle (Bmal1 → Wee1)
- **Almost no knowledge** on how cell cycle affects clock (at mitosis gene expression shuts down)

Cell cycle and circadian rhythms
How do they interact?

Gating
(cell division allowed only at certain clock phases)

Independent?

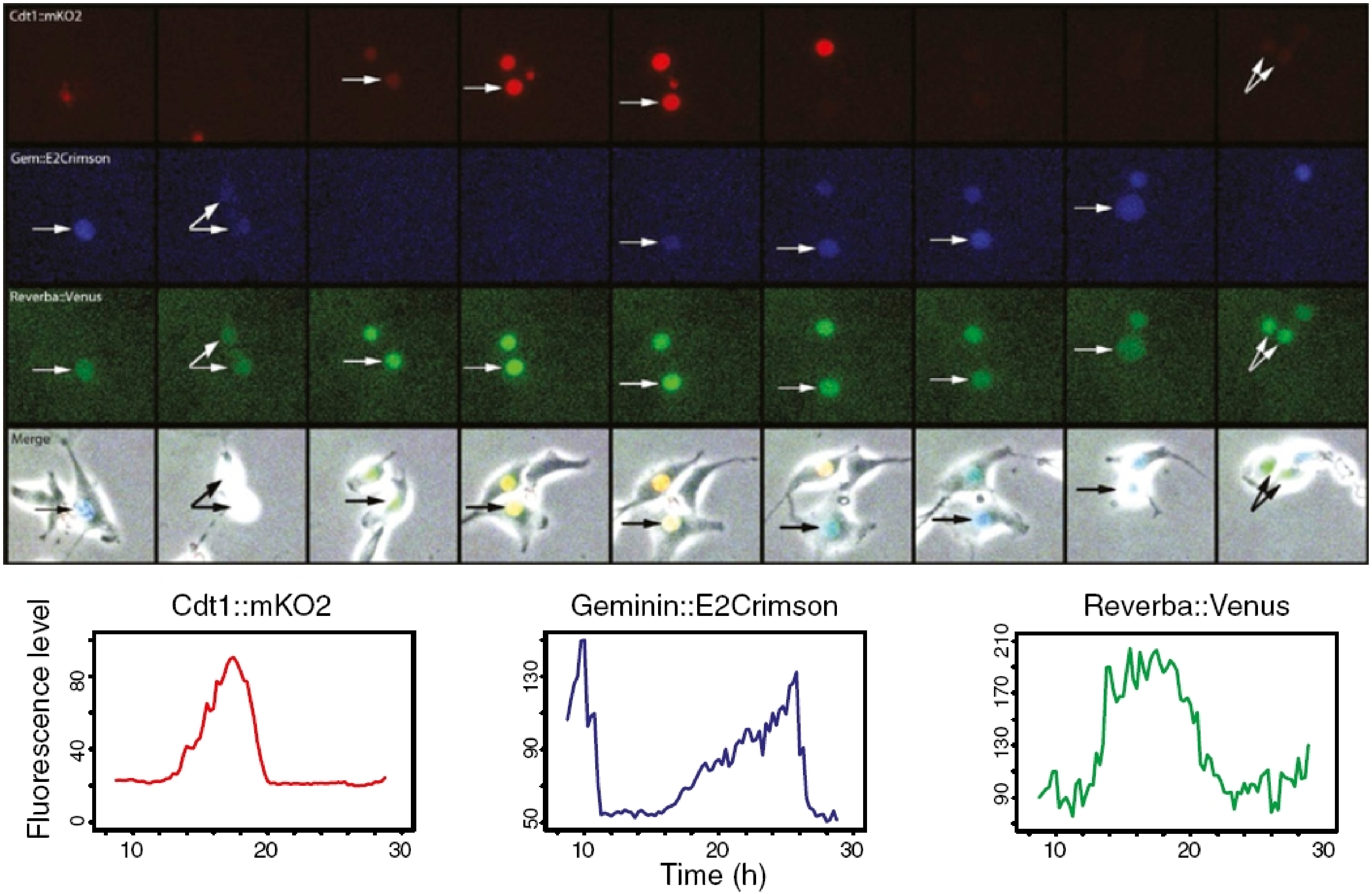
Coordinated?

Observed in Cyanobacteria

No gating in mammals

Mammalian clock and cell cycle experiments

Feuillet et al, PNAS 2014 (Delaunay Lab)   (Cells: mouse fibroblasts)



Red: cell cycle, G1 phase
cells are growing

Blue: cell cycle, G2/S phase
cells are getting ready to divide

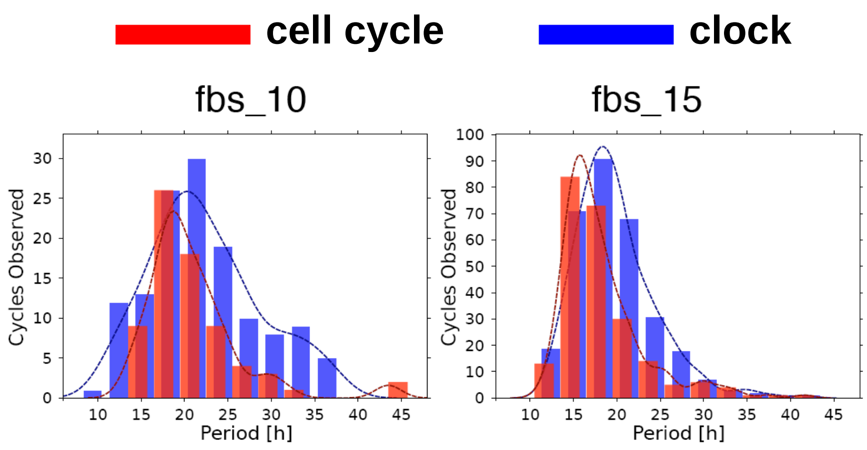
Green: clock, high phase
right after division

Mammalian clock and cell cycle experiments

Feuillet et al, PNAS 2014 (Delaunay Lab)   (Cells: mouse fibroblasts)

Change the cell cycle period ?

Apply different concentrations of growth hormone



– cell cycle period decreases

– clock decreases by same amount

– 1:1 lock

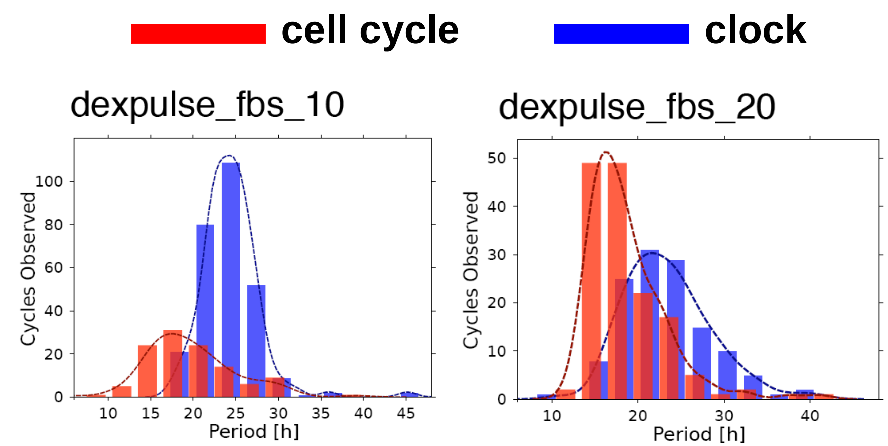
Evidence in favour of: **cell cycle** → **clock**

Mammalian clock and cell cycle experiments

Feuillet et al, PNAS 2014 (Delaunay Lab)   (Cells: mouse fibroblasts)

Change the clock period ?

Apply a drug that synchronizes cells clock, dexamethasone



– synchronized cells

– two subpopulations (20% fbs):

1:1   lock

3:2   (cc:clock)

Evidence in favour of: **cell cycle** ← **clock**

How to identify the coupling mechanism ?

Mammalian
Clock +
Cell cycle
Too complex!

How to identify the coupling mechanism ?
→ SYNTHETIC BIOLOGY APPROACH

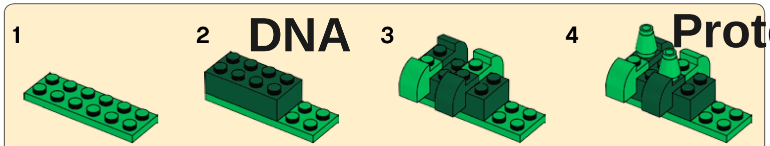
collaboration
F. Delaunay Lab

Mammalian
Clock +
Cell cycle
Too complex!

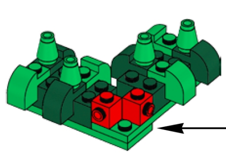
↑

**Synthetic
Oscillators,
Biological bricks**

DNA **Proteins**



**Activations,
Inhibitions**



How to identify the coupling mechanism ?
→ DYNAMICAL ANALYSIS & PREDICTIONS

Mammalian Clock + Cell cycle Too complex!

→

Mathematical Models Too large!

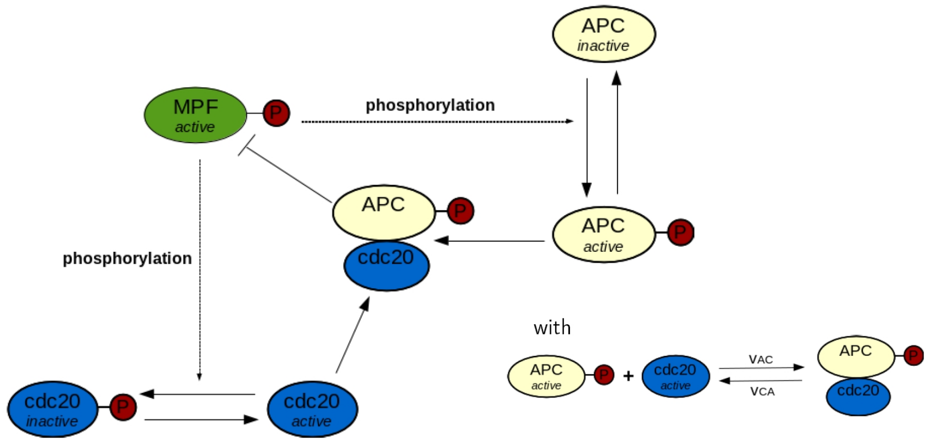
↓

Model reduction & Interconnections

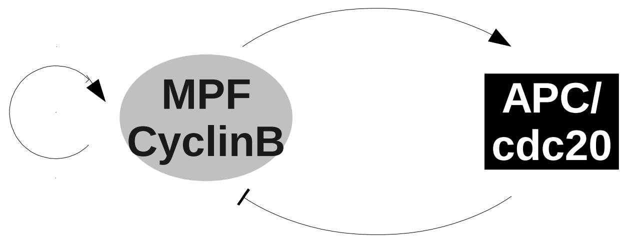
←

Synthetic Oscillators, Biological bricks

↑

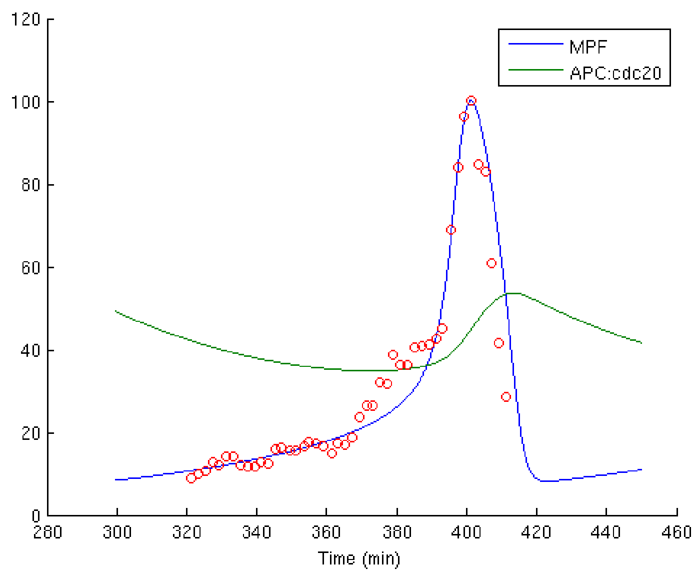


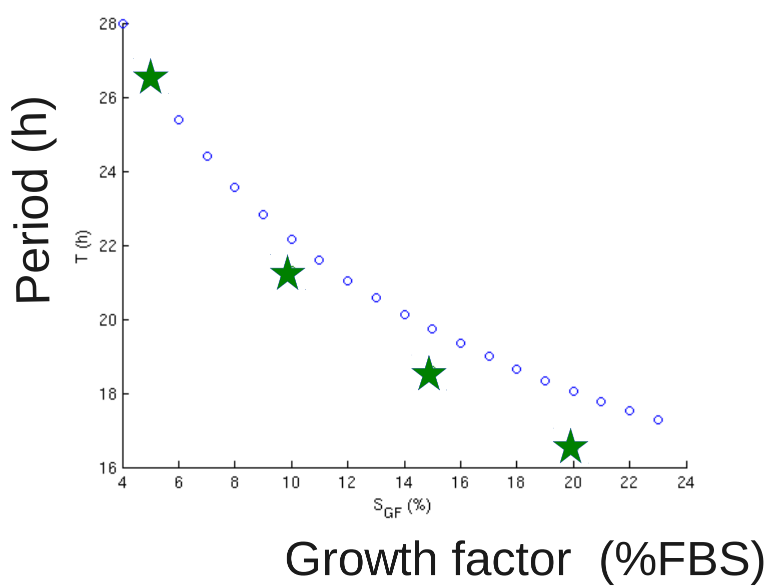
Reduced cell cycle model



○ **Model predictions**
(on growth factor)

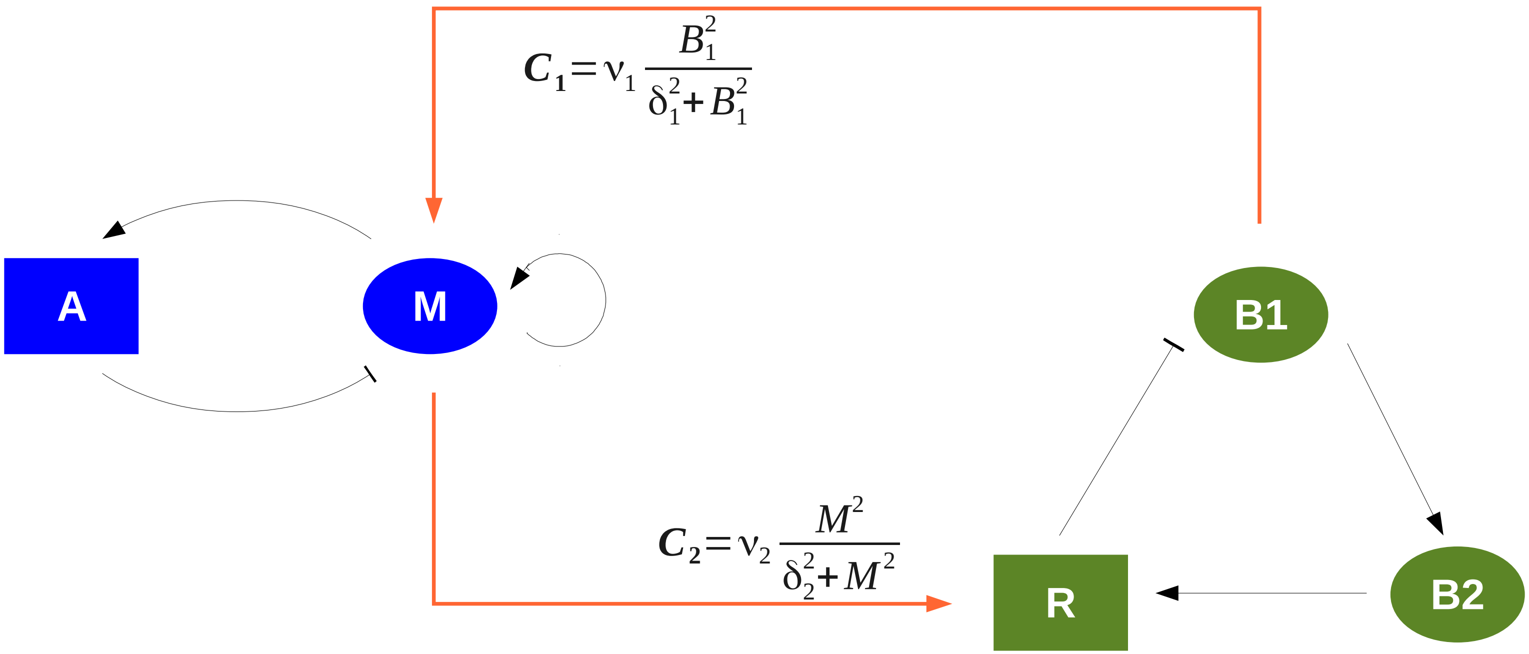
★ **Experimental data**
(fetal bovine serum)
(F. Delaunay lab)





S. Almeida et al, IFAC 2017

Interconnecting cell cycle and clock



Interconnecting cell cycle and clock

- Each oscillator has a different “individual” period
- When coupled, the two oscillators synchronize 1:1 (or stop oscillating)
- Question 1: **range of periods** over which the two oscillators synchronize?
- Question 2: is an oscillator either **a “controller” or a “follower”** ?

  ie., is the final period closer to the clock or cell cycle individual periods?
- Characterize dependence of these observations on the coupling strength

In summary

- Cell cycle and circadian rhythms: Are they interconnected? And how? Many questions ! A current and lively topic

- Synthetic biology approach: minimal circuit design with desired properties

- Our tools: model reduction, mathematical analysis, and control

Merci !

SIGNALIFE institut Valrose Biologie UNIVERSITÉ CÔTE D'AZUR Inria informatiques mathématiques AGENCE NATIONALE DE LA RECHERCHE ANR