L'horloge circadienne, le cycle cellulaire et leurs interactions

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What do all these living organisms have in common?

- Cyanobacteria in Baltic sea
- Neurospora crassa
- Arabidopsis thaliana
- Mus musculus
- Drosophila melanogaster
- Synechococcus elongatus
- Cyanobacteria in Baltic sea
Cell cycle

- Cell cycle is the process by which cells grow 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

Cortisol
Lipid degradation, Glucose production (energy)

Melatonin
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
Negative feedback loop

OFF  OFF

Per mRNA  PER protein
Negative feedback loop

PER protein

Per mRNA

OFF OFF

ON OFF
Negative feedback loop

OFF OFF

ON OFF

ON ON

Per mRNA

PER protein
Negative feedback loop

Generates an oscillatory Cycle!
Negative feedback loop

Per mRNA 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

**Drosophila melanogaster**

**Arabidopsis thaliana**

**Neurospora crassa**

**Mammalian clock**

Nature Reviews | Cancer
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**

**Closed circles:** Basin of attraction of $x^*$

**Open circles:** Basin of attraction of periodic orbit

**Shaded:** Invariant Region (PWA)

**Prediction:** there exist critical points in the periodic orbit, where the system may be perturbed and the circadian cycle arrested – such as when adding a large amount of Kai A.
Model predictions: response to light

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 $\text{ATP/(ATP+ADP)}$. 

Chaves & Preto, Chaos 2013
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

Independent?
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

Evidence in favour of:

- synchronized cells
- two subpopulations (20% fbs):
  1:1  lock
  3:2  (cc:clock)
How to identify the coupling mechanism?

- Mammalian Clock + Cell cycle
- Too complex!
How to identify the coupling mechanism?

→ **SYNTHETIC BIOLOGY APPROACH**

1. Mammalian Clock + Cell cycle
2. Too complex!
3. Synthetic Oscillators, Biological bricks
4. DNA
5. Proteins

**Activations, Inhibitions**

Collaboration
F. Delaunay Lab
How to identify the coupling mechanism?
→ DYNAMICAL ANALYSIS & PREDICTIONS

Mammalian Clock + Cell cycle
Too complex!

Synthetic Oscillators, Biological bricks

Mathematical Models
Too large!

Model reduction & Interconnections

How to identify the coupling mechanism?
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

\[ C_1 = \nu_1 \frac{B_1^2}{\delta_1^2 + B_1^2} \]

\[ C_2 = \nu_2 \frac{M^2}{\delta_2^2 + M^2} \]
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”?
  
i.e., 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!