# Circadian rhythms

# Biological rhythms

| Biological rhythm | Period |
|---|---|
| Neural rhythms* | 0.001 s to 10 s |
| Cardiac rhythm* | 1 s |
| Calcium oscillations* | sec to min |
| Biochemical oscillations* | 30 s to 20 min |
| Mitotic oscillator* | 10 min to 24 h |
| Hormonal rhythms* | 10 min to 3-5 h (24 h) |
| Circadian rhythms* | 24 h |
| Ovarian cycle | 28 days (human) |
| Annual rhythms | 1 year |
| Rhythms in ecology and epidemiology | years |

*Cellular rhythms

**Biological rhythms** are observed at various levels of the organization of all living systems.

They display a variety of shapes (sine, spike, burst, etc) and periods.

They are generated through diverse mechanisms.

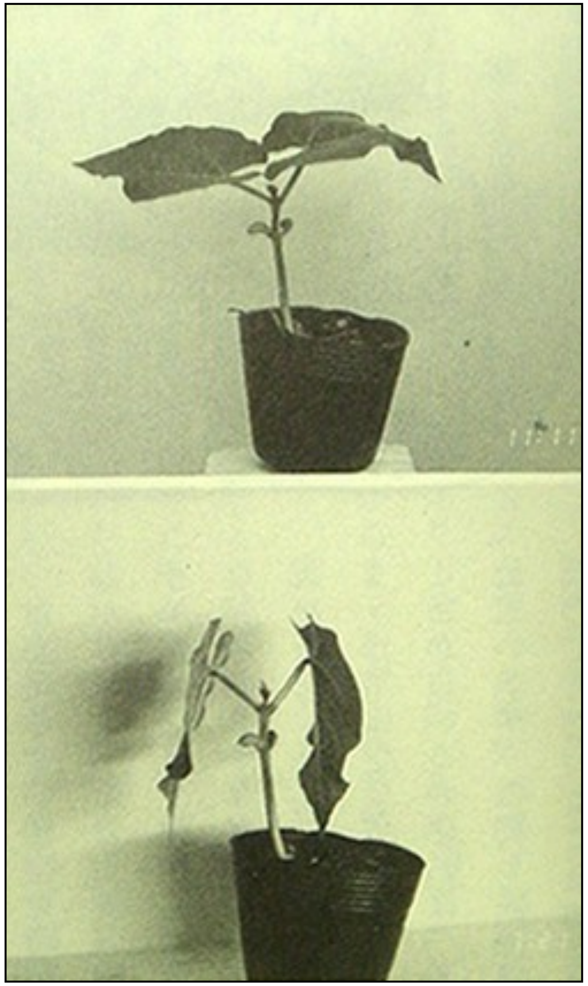
**Modeling** help to unravel the mechanisms underlying these biological rhythms

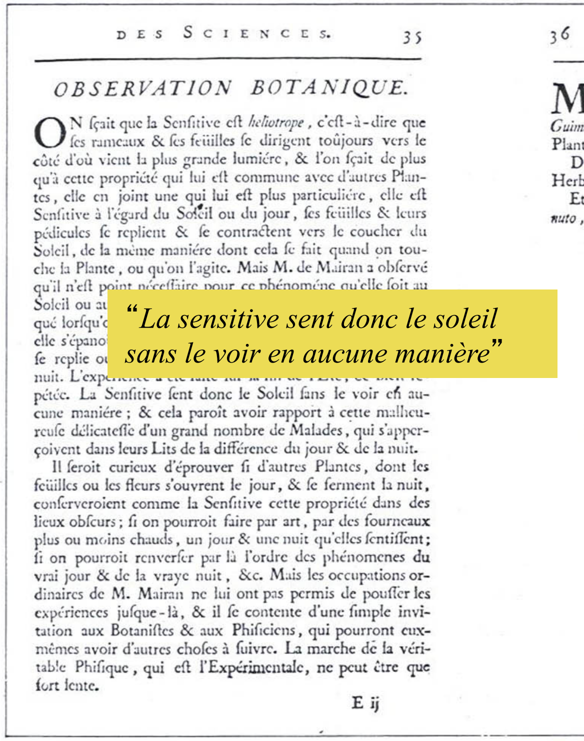
circadian clocks
(molecular mechanism)

*Circadian rhythms are biological rhythms with the longest period originating at the cellular level.*

# Circadian rhythms

**Circadian rhythms** are **endogeneous** biological rhythms characterized by a period of **about 24h**...







*OBSERVATION BOTANIQUE.*

ON ſçait que la Senſitive eſt *heliotrope*, c'eſt-à-dire que ſes rameaux & ſes feüilles ſe dirigent toûjours vers le côté d'où vient la plus grande lumiére, & l'on ſçait de plus qu'à cette propriété qui lui eſt commune avec d'autres Plantes, elle en joint une qui lui eſt plus particuliére, elle eſt Senſitive à l'égard du Soleil ou du jour, ſes feüilles & leurs pédicules ſe replient & ſe contractent vers le coucher du Soleil, de la même maniére dont cela ſe fait quand on touche la Plante, ou qu'on l'agite. Mais M. de Mairan a obſervé qu'il n'eſt point néceſſaire pour ce phénoméne qu'elle ſoit au Soleil ou au [...] qué lorſqu'e[...] elle s'épano[...] ſe replie o[...] nuit. L'exp[...] pétée. La Senſitive ſent donc le Soleil ſans le voir en aucune maniére; & cela paroît avoir rapport à cette malheureuſe délicateſſe d'un grand nombre de Malades, qui s'apperçoivent dans leurs Lits de la différence du jour & de la nuit.

Il ſeroit curieux d'éprouver ſi d'autres Plantes, dont les feüilles ou les fleurs s'ouvrent le jour, & ſe ferment la nuit, conſerveroient comme la Senſitive cette propriété dans des lieux obſcurs; ſi on pourroit faire par art, par des fourneaux plus ou moins chauds, un jour & une nuit qu'elles ſentiſſent; ſi on pourroit renverſer par là l'ordre des phénomenes du vrai jour & de la vraye nuit, &c. Mais les occupations ordinaires de M. Mairan ne lui ont pas permis de pouſſer les expériences juſque-là, & il ſe contente d'une ſimple invitation aux Botaniſtes & aux Phiſiciens, qui pourront eux-mêmes avoir d'autres choſes à ſuivre. La marche de la véritable Phiſique, qui eſt l'Expérimentale, ne peut être que fort lente.





M. Marchant a lû la Deſcription

De l'*Althoea* Dioſc. & Plin. C. B. Pin. 315. *Guimauve*, avec la Critique des Auteurs Botaniſtes ſur cette Plante.

De la *Mitella Americana, florum foliis fimbriatis*. Inſt. Raii Herb. 242.

Et de la *Sanicula*, ſeu *Cortuſa Americana*, *altera*, *flore minuto*, *fimbriato*. Hort. Reg. Par.

*"La sensitive sent donc le soleil sans le voir en aucune manière"*

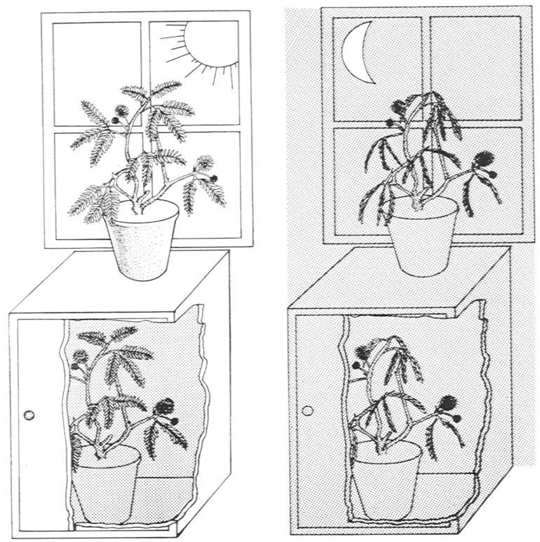
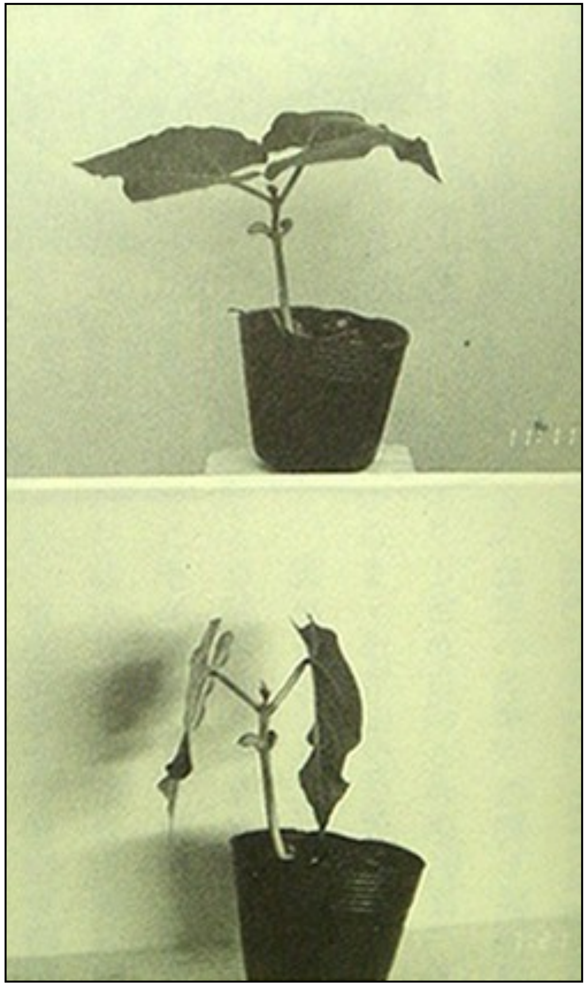


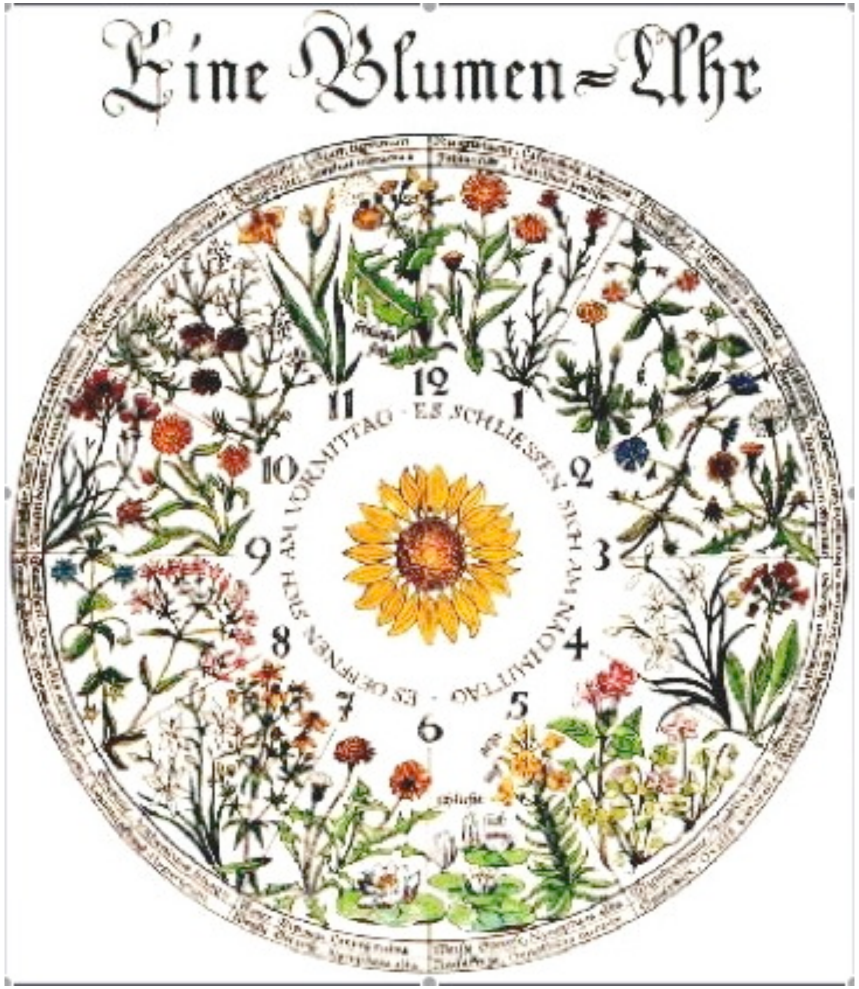
**Fig. 1.4** A representation of de Mairan's original experiment. When exposed to sunlight during the day (*upper left*), the leaves of the plant were open, and during the night (*upper right*) the leaves were folded. De Mairan showed that sunlight was not necessary for these leaf movements by placing the plant in total darkness; even under these constant conditions, the leaves opened during the day (*lower left*) and folded during the night (*lower right*). (Copyright 1982 by Moore-Ede, Sulzman, and Fuller.)

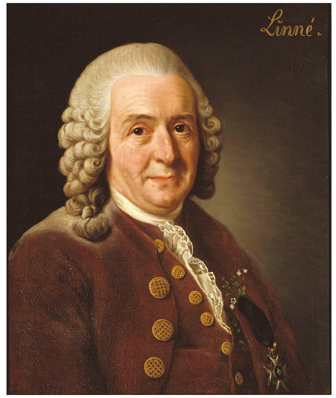
**Jean-Jacques d'Ortous de Mairan (1740)**

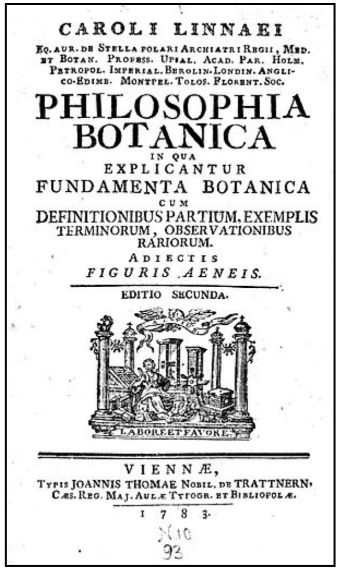
# Circadian rhythms

**Carl von Linné** (1751, *Philosophia Botanica*) imagined a circular garden composed of plants that open and close their flowers at particular times of the day, displayed such that we could read the time (**flower clock**).





CAROLI LINNAEI

EQ. AUR. DE STELLA POLARI ARCHIATRI REGII, MED. ET BOTAN. PROFESS. UPSAL. ACAD. PAR. HOLM. PETROPOL. IMPERIAL. BEROLIN. LONDIN. ANGLICO-EDIMB. MONTPEL. TOLOS. FLORENT. SOC.

PHILOSOPHIA BOTANICA

IN QUA EXPLICANTUR FUNDAMENTA BOTANICA CUM DEFINITIONIBUS PARTIUM, EXEMPLIS TERMINORUM, OBSERVATIONIBUS RARIORUM.

ADIECTIS FIGURIS AENEIS.

EDITIO SECUNDA.

LABORE ET FAVORE.

VIENNÆ,

TYPIS JOANNIS THOMAE NOBIL. DE TRATTNERN, CÆS. REG. MAJ. AULÆ TYPOGR. ET BIBLIOPOLÆ.

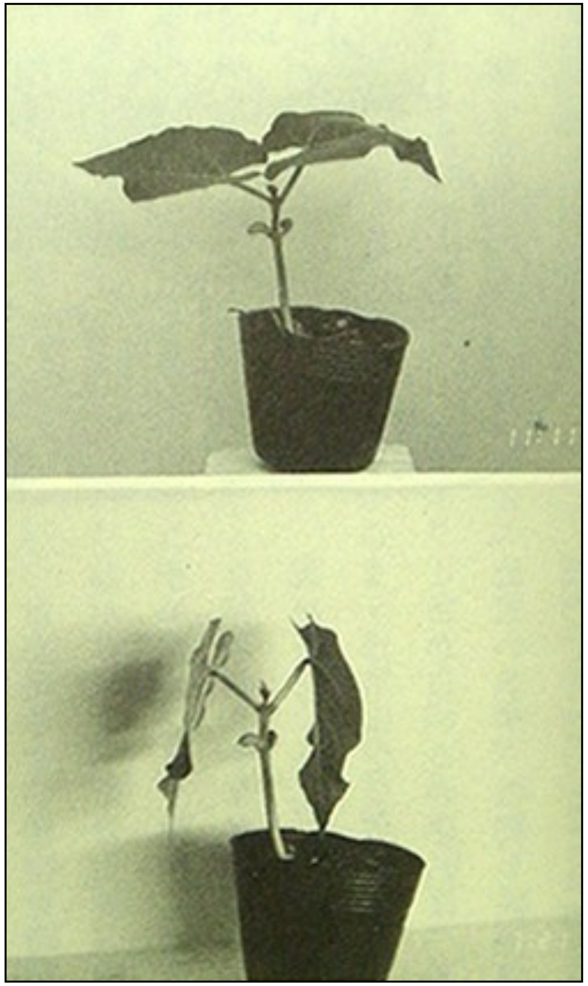
1783.

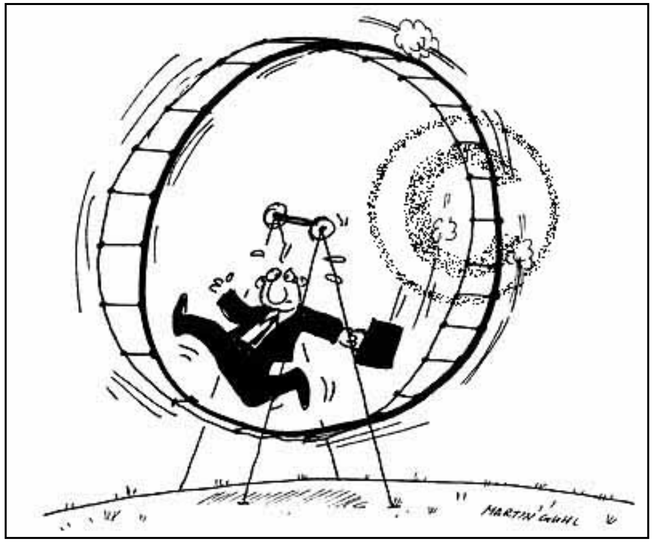
N 10

93

# Circadian rhythms

**Circadian rhythms** are **ubiquitous**. They allow living organisms to live in phase with the alternance of day and night...





# Circadian rhythms

**Our biological clock regulates many physiological functions:** Blood pressure, Body temperature, Hormone secretion, Sleep-wake cycle, Heart beat, Metabolism, Cell cycle, Response to drugs,...

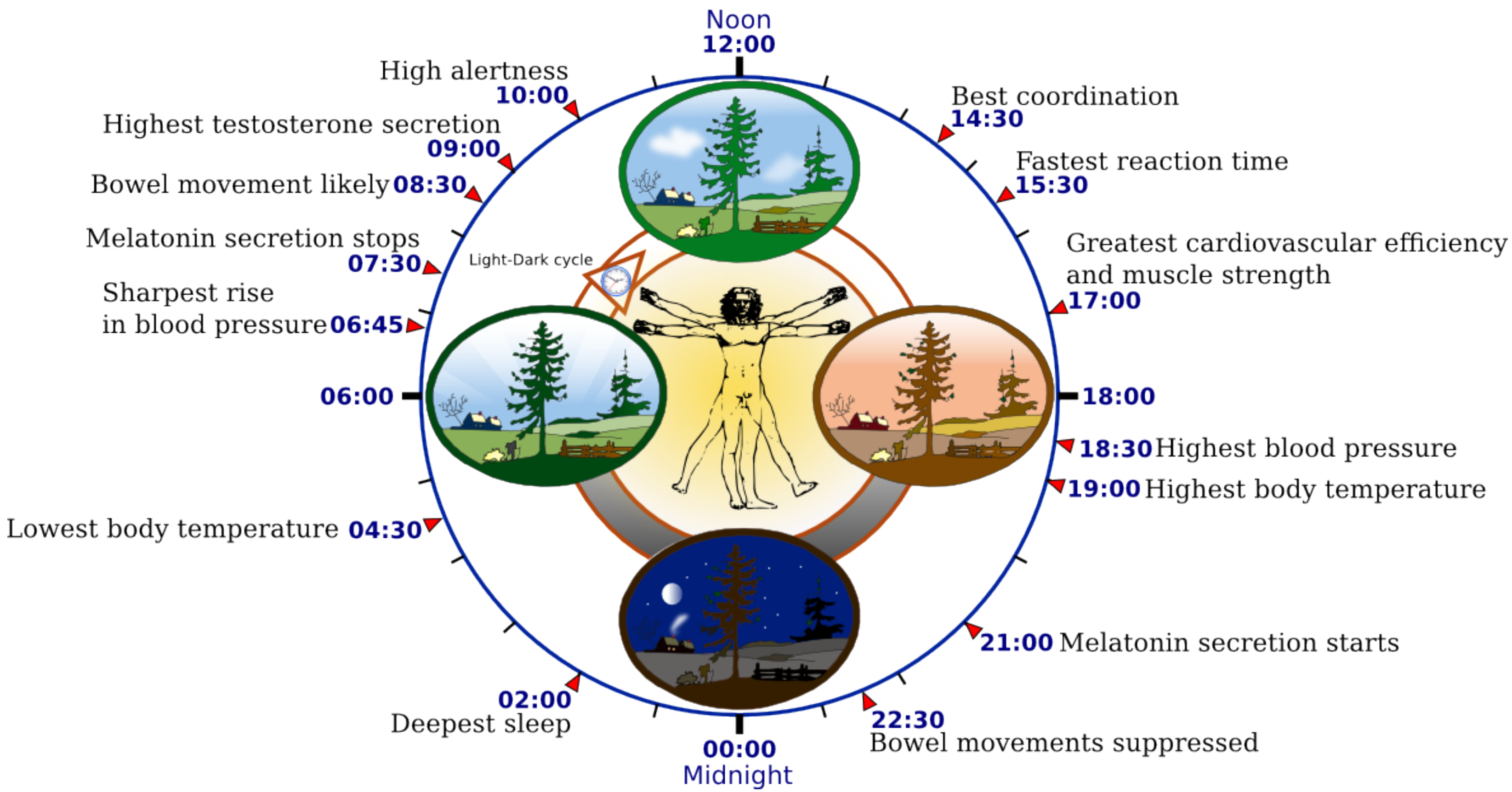



Figure: wikipedia

# Circadian rhythms

**Circadian rythms have been "measured" in most organs:**

- Brain (SCN, pineal gland, etc)
- Lung
- Heart
- Liver
- Kidney
- Fibroblasts
- ...

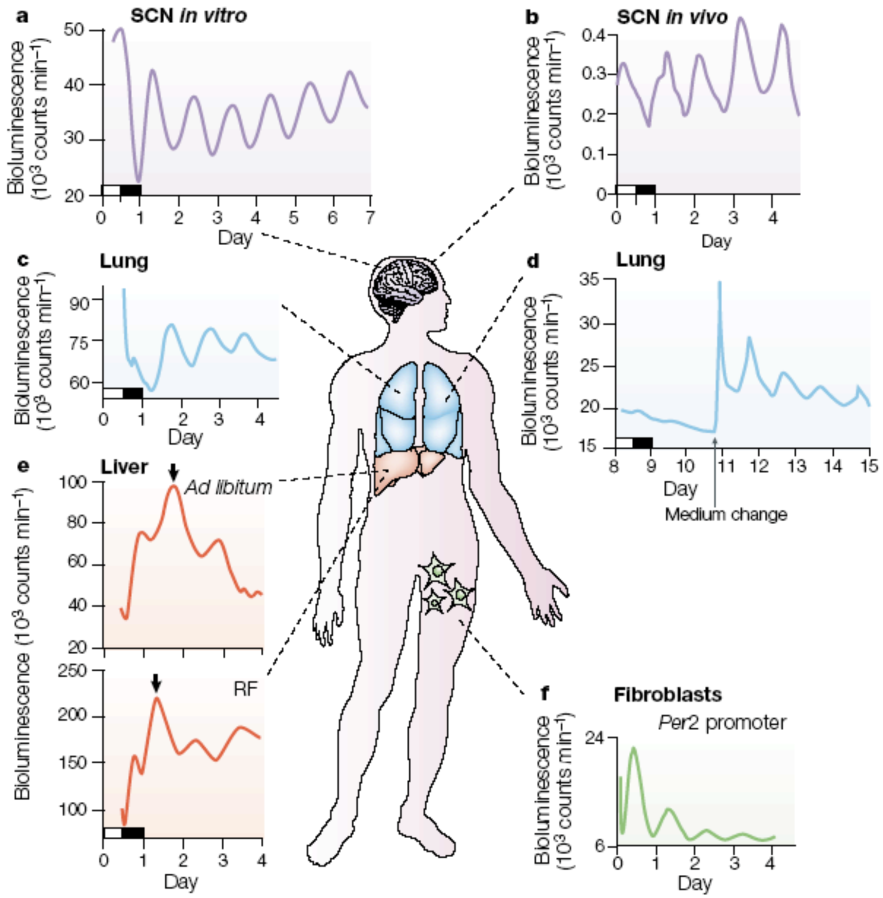
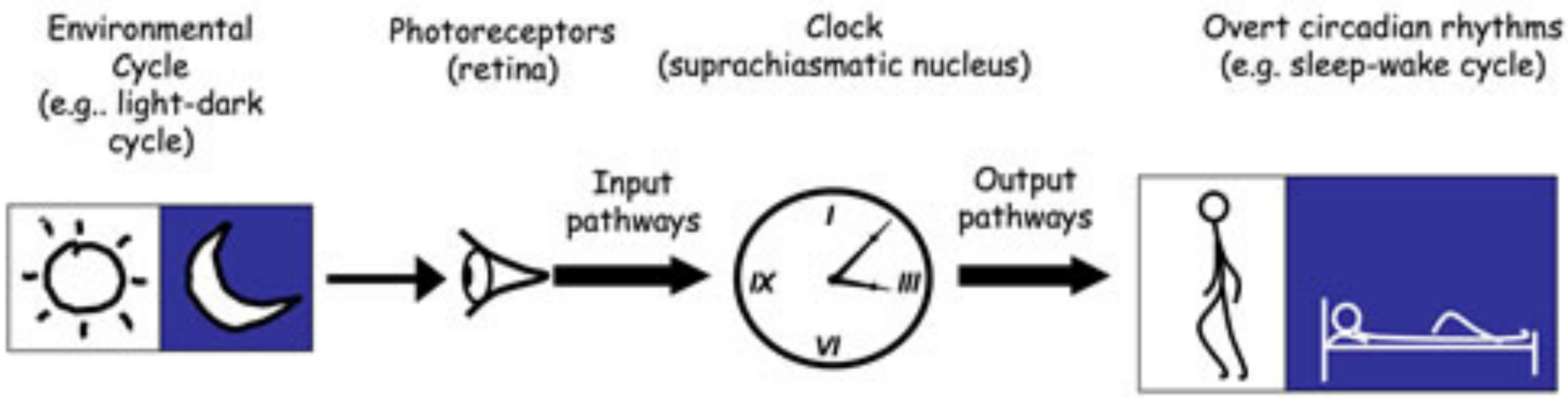



Figure 3 | *Per::luciferase* **transgenes reveal a diversity of tissue-based circadian oscillators.**

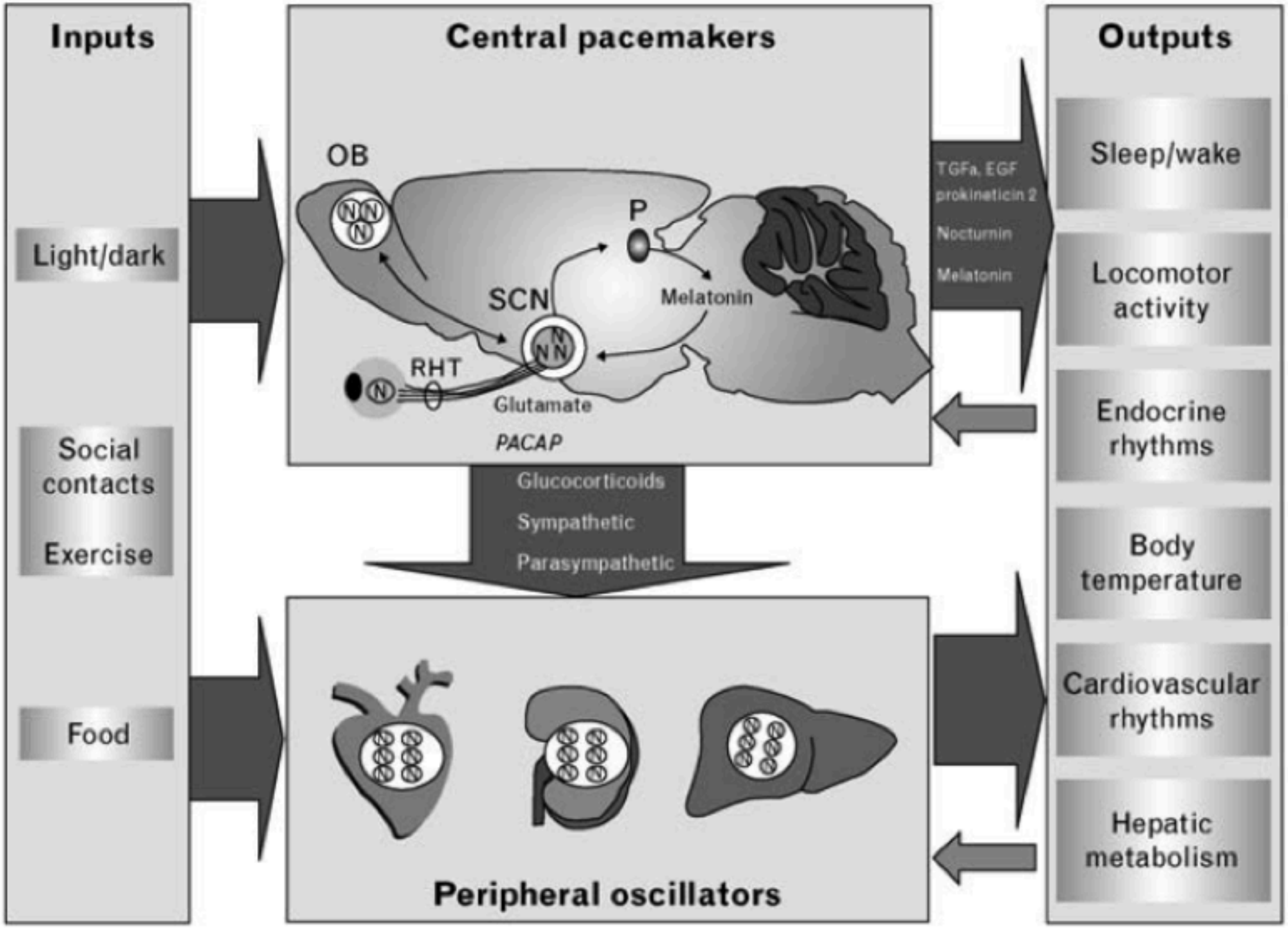
Hastings (2003) *Nat Rev Neurosci 4*: 649-61.

# Circadian rhythms

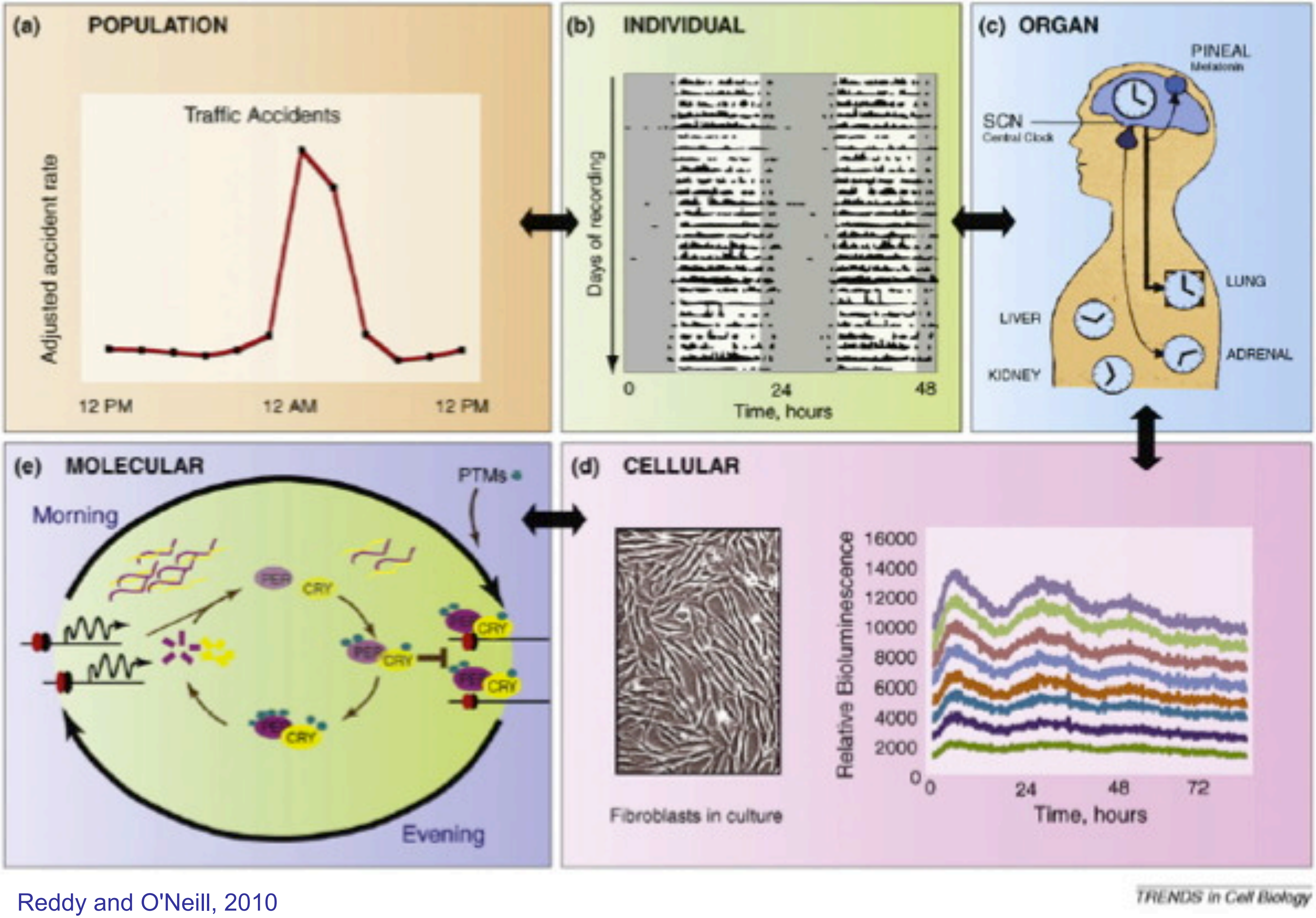



Source: Horacio de la Iglesia (http://www.fac.org.ar/qcvc/llave/c110i/iglesiah.php)

# Circadian rhythms



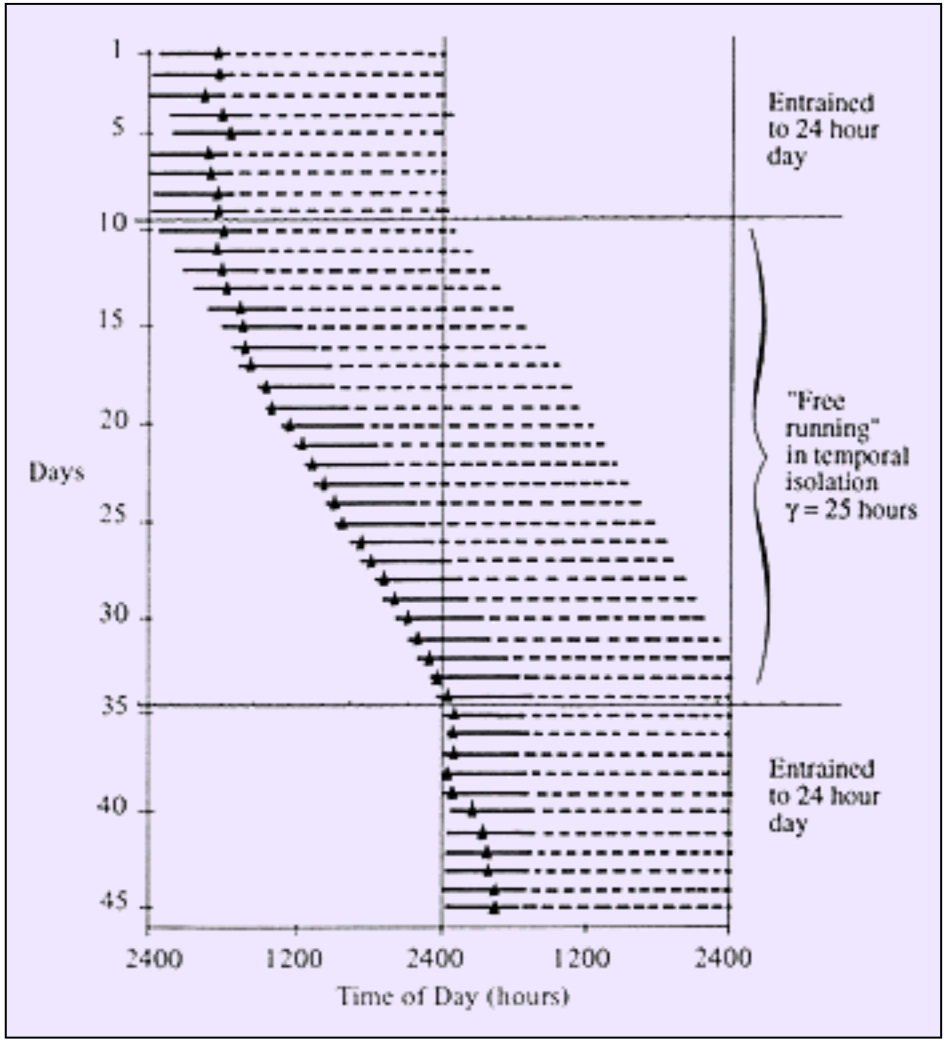
# Circadian rhythms




Reddy and O'Neill, 2010

# Circadian rhythms

## Free-run vs entrainment




- In **constant conditions**, the *free-running period* of circadian rhythm is *around* 24h (but not exactly 24h)

**Franz Halberg (born in 1919)**

*Father of Chronobiology*

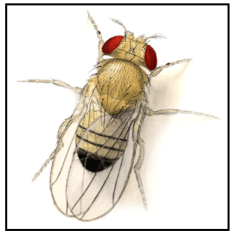
Coined the term *circadian* (1959)

circa (around) — dies (day)

- In **natural conditions** circadian rhythms are *entrained* by the external light-dark cycle and the period is *exactly* 24h.

# Circadian rhythms

## Monitoring the locomotor activity of flies (wild type and perS mutant)





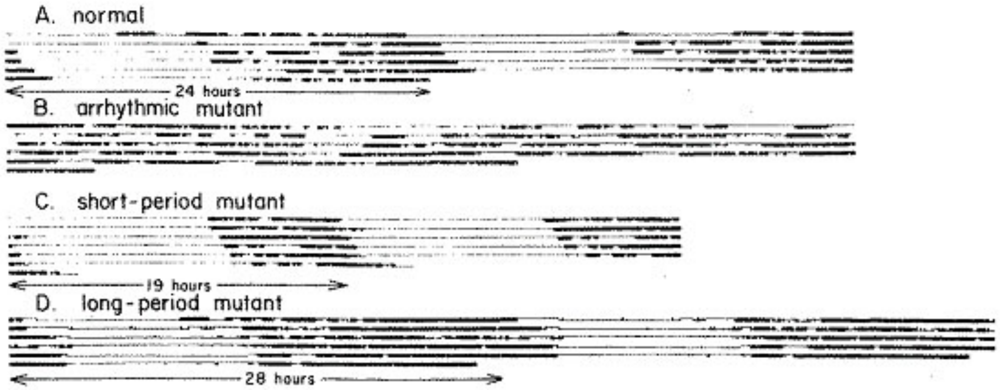
See animations: droso_activity_WT.avi and droso_activity_PERS.avi

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

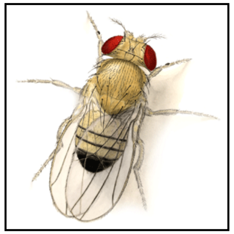
Source: http://www.hhmi.org/biointeractive/clocks/animations.html

# Circadian rhythms: genetic bases

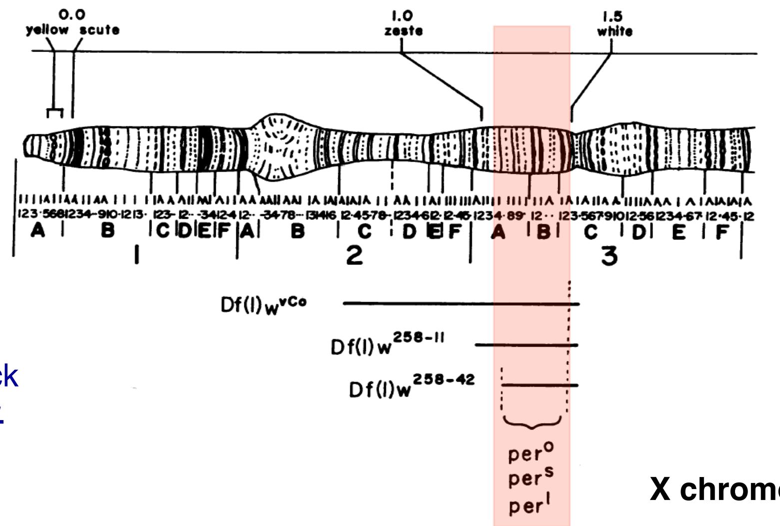
**Locomotor activity (actogram)**




*Drosophila*



**Identification of the *period* (*per*) gene**




**X chromosome**

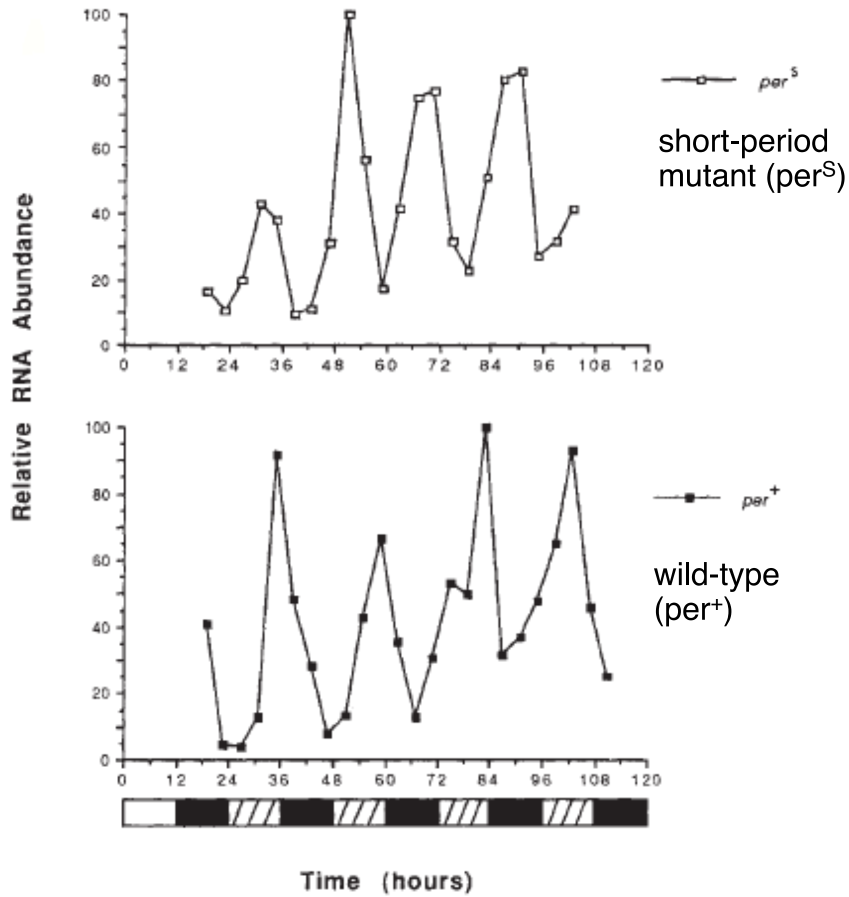
Konopka RJ & Benzer S (1971) Clock mutants of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 68, 2112-6.

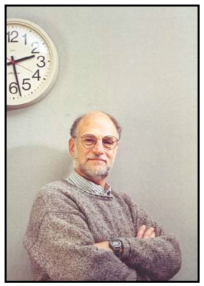
# Circadian rhythms: genetic bases

## Oscillations of *per* mRNA and PER protein in *Drosophila*

- Levels of *per* mRNA and PER proteins oscillates with a circadian period (both in the eye and in the brain of the fly).
- The peak of PER protein occurs a few hours after the peak of mRNA.
- The fact that mRNA level decreases when the level of protein is high (together with other experiments) suggests that the PER protein inhibits the expression of the *per* gene.

**Oscillations of *per* mRNA in constant darkness**

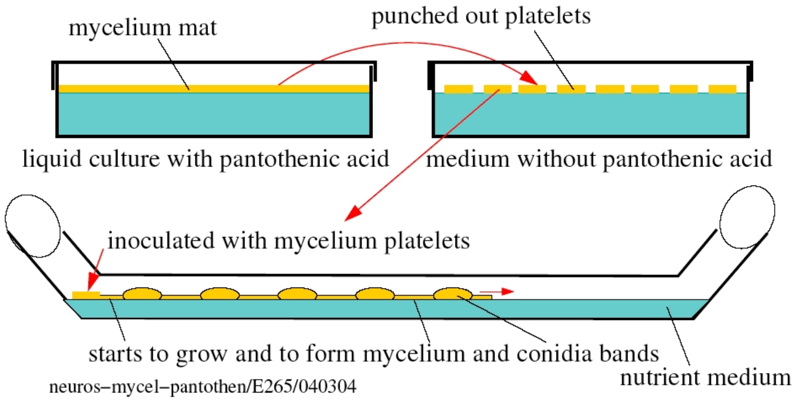


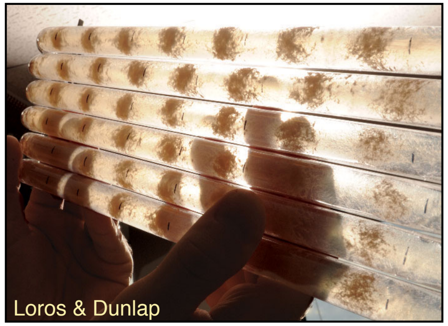
Zeng H, Hardin PE, Rosbash M (1994) Constitutive overexpression of the *Drosophila* period protein inhibits *period* mRNA cycling. *EMBO J.* 13: 3590-3598.

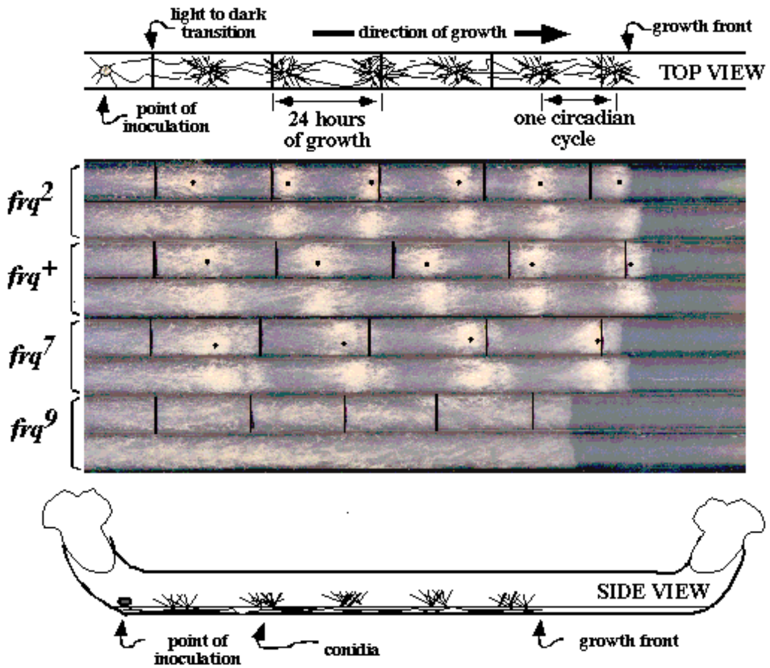
# Circadian rhythms: genetic bases

## Circadian rhythm of conidiation in *Neurospora*

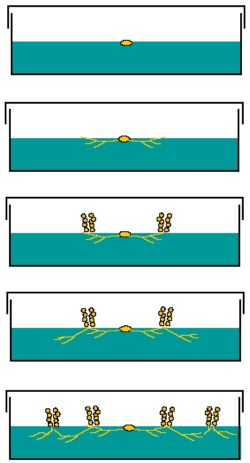


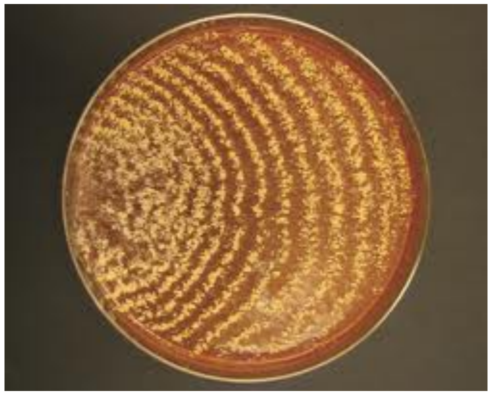

race tube




Petri dish

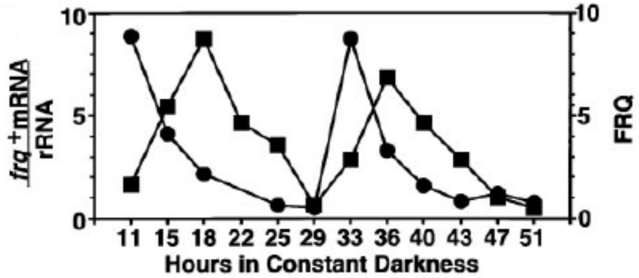




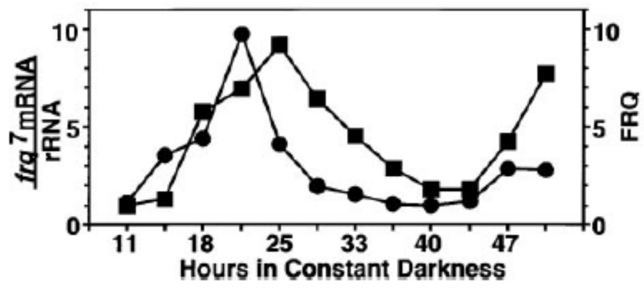
# Circadian rhythms: genetic bases

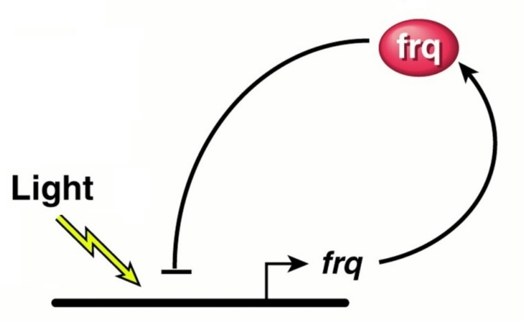
## Oscillations of *frq* mRNA and FRQ protein in *Neurospora*

***frq*+ (wild type)**




***frq*7 (long-period mutant)**



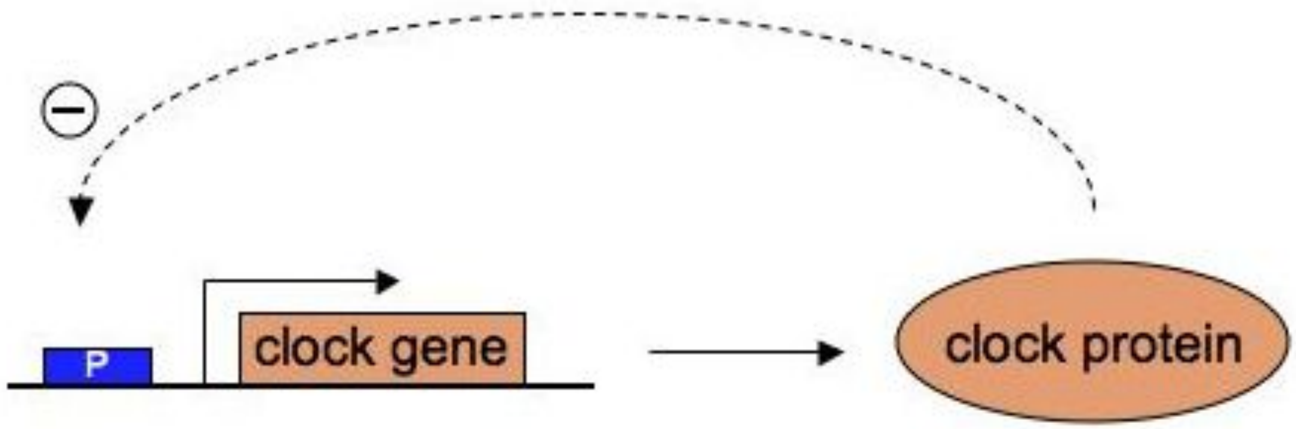

The core mechanism of circadian oscillations relies on a negative transcriptional feedback loop

- In *Drosophila*, the protein PER inhibits the expression of its own gene, *per*.
- In *Neurospora*, the protein FRQ inhibits the expression of its own gene, *frq*.


Garceau NY, Liu Y, Loros JJ, Dunlap JC (1997) Alternative initiation of translation and time-specific phosphorylation yield multiple forms of the essential clock protein FREQUENCY. Cell. 89:469-76.
Aronson BD, Johnson KA, Loros JJ, Dunlap JC (1994) Negative feedback defining a circadian clock: autoregulation of the clock gene frequency. Science. 263:1578-84.

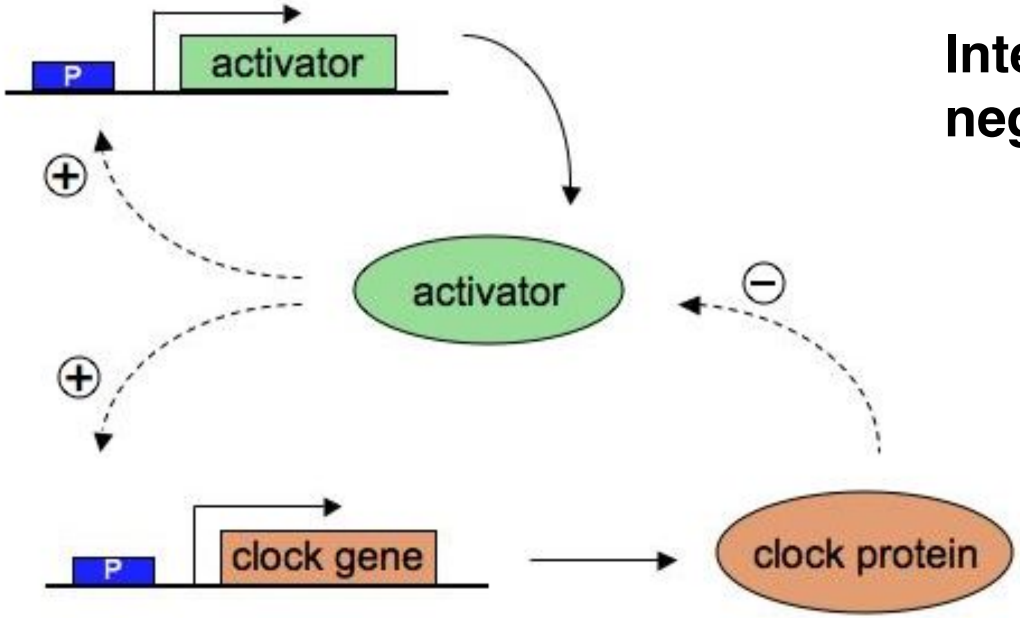

# Molecular mechanism of circadian clocks

## Core mechanism: negative feedback loop




| | clock gene |
|---|---|
| *Drosophila* | ***per*** *(period),* ***tim*** *(timeless)* |
| *Mammals* | ***mper1-3*** *(period homologs)* |
| *Neurospora* | ***frq*** *(frequency)* |

# Molecular mechanism of circadian clocks




**Interlocked positive and negative feedback loops**

| | Clock gene | Activator | Effect of light |
|---|---|---|---|
| *Drosophila* | *per, tim* | *clk, cyc* | TIM degradation |
| *Mammals* | *mper1-3, cry1,2* | *clock, bmal1* | *per* transcription |
| *Neurospora* | *frq* | *wc-1, wc-2* | *frq* transcription |


Dunlap JC (1999) Molecular bases for circadian clocks. *Cell* 96: 271-290.
Young MW & Kay SA (2001) Time zones: a comparative genetics of circadian clocks. *Nat. Genet.* 2: 702-715.

# Molecular mechanism of circadian clocks

## Circadian clock in mammals

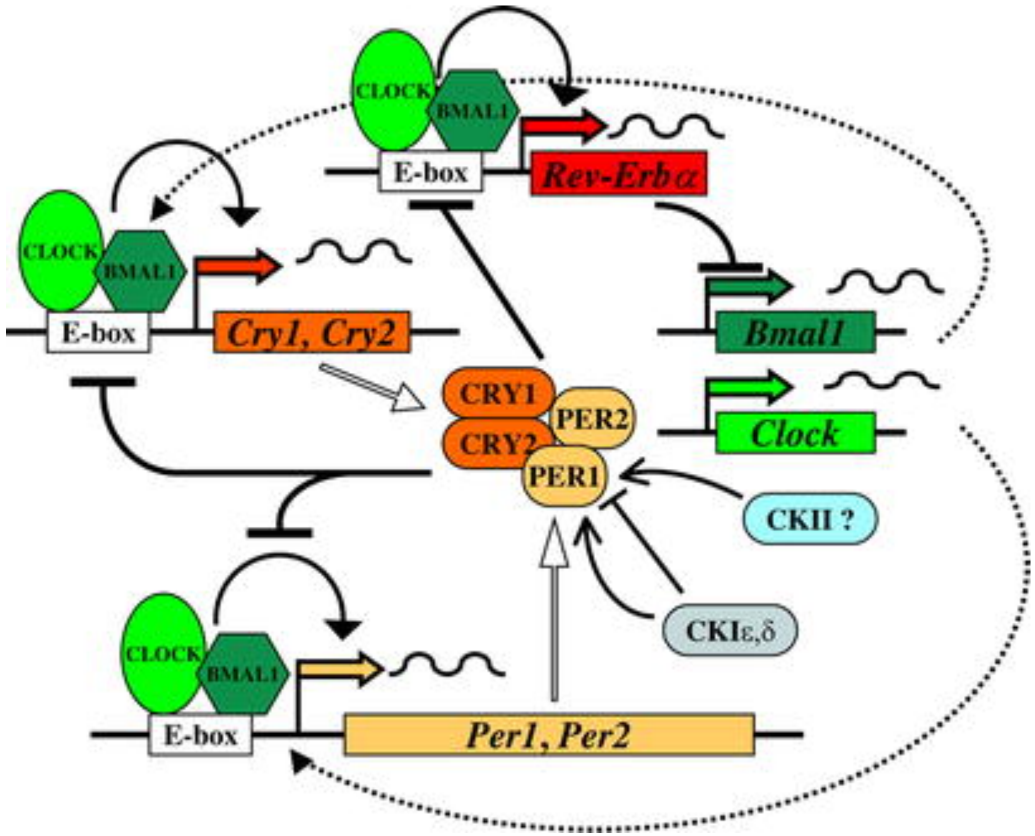


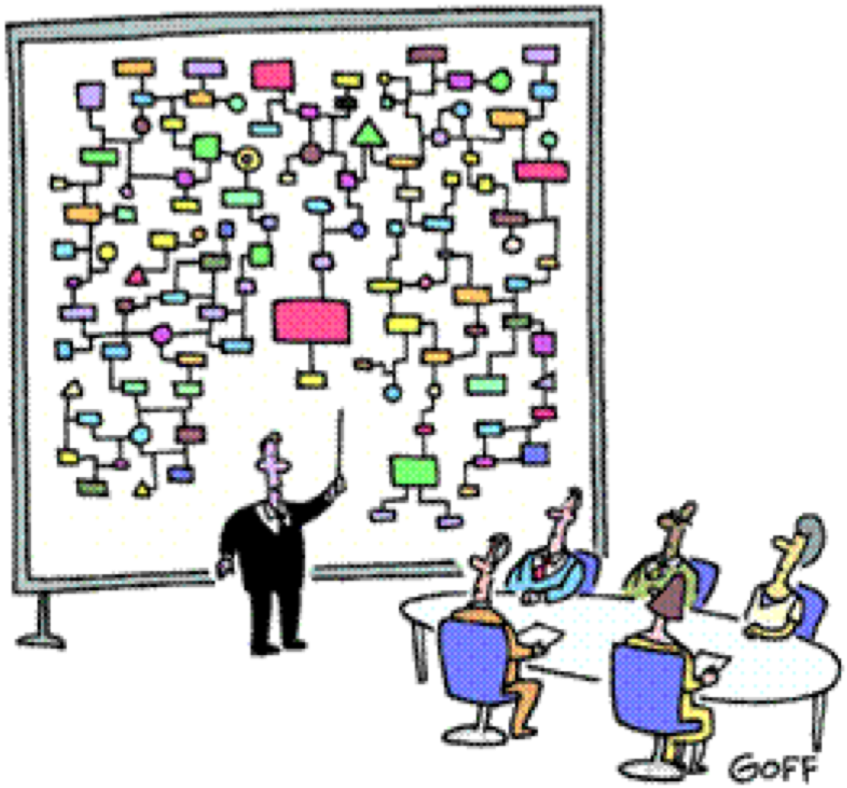

Figure from Gachon, Nagoshi, Brown, Ripperger, Schibler (2004) The mammalian circadian timing system: from gene expression to physiology. *Chromosomia* 113: 103-112.

# Molecular mechanism of circadian clocks

## Properties of circadian clocks

- autonomous oscillator (endogeneous clock)
- period ≈ 24h (circadian) in free-running condition
- temperature compensation
- robust to noise
- integrates multiple inputs (light, temperature, food, etc)
- controls many physiological processes
- genetic basis (interlocked feedback loops)
- intercellular coupling and synchronization (in the SCN)
- inter-organ coupling (pacemaker /peripheral clocks)

# Modeling circadian clocks




"And that's why we need a computer."

# Modeling circadian clocks

## Roles and advantages of modeling

- Analysing and understanding complex situations that become difficult to describe in verbal terms and for which sheer intuition becomes unreliable.
- Rapid exploration of different mechanims and large ranges of conditions.
- Identification of key interactions and parameters, and their qualitative or quantitative influence for the system's behaviour.
- Determine precisely the conditions in which different behaviours will occur.
- To address questions that are difficult or impossible to approach experimentally.
- Provide testable predictions, suggestions for new experiments or model modifications, and sometimes counterintuitive explanations that may corroborate or not conclusions drawn from experimental observations.
- Provide a unified framework to account for the experimental observations and bring into light possible similarities between apparently unrelated processes.

Leloup J-C and Goldbeter A (2000) Modeling the molecular regulatory mechanism of circadian rhytms in Drosophila. BioEssays 22, 83-92
Gonze D (2011) Modeling circadian clocks: Roles, advantages, and limitations, Cent. Eur. J. Biol. 6:712-729

# First models for circadian clocks

## Pavlidis model

$$\frac{dr}{dt} = r + a_0 - a_1 s - a_2 s^2 - K \cdot L$$

$$\frac{ds}{dt} = r - as, \quad (r \geq 0)$$

Model that takes into account the effect of light (L=light intensity, K=sensitivity to light).

The equations are arbitrary but have been chosen to generate limit cycle oscillations and to reproduce the phase response curve.

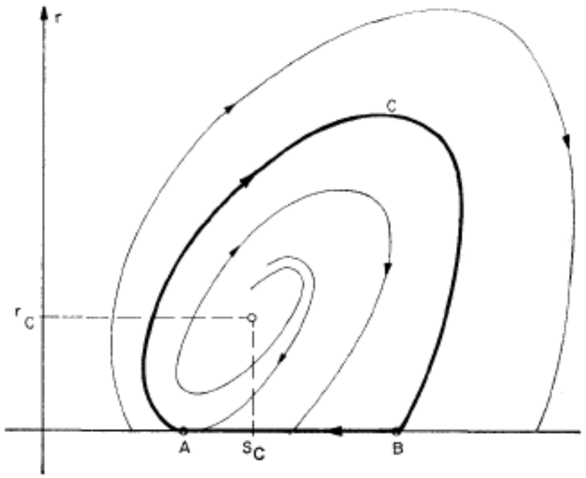



Figure 1. Phase plane plot of the trajectories of the system of equations (1) and (2)

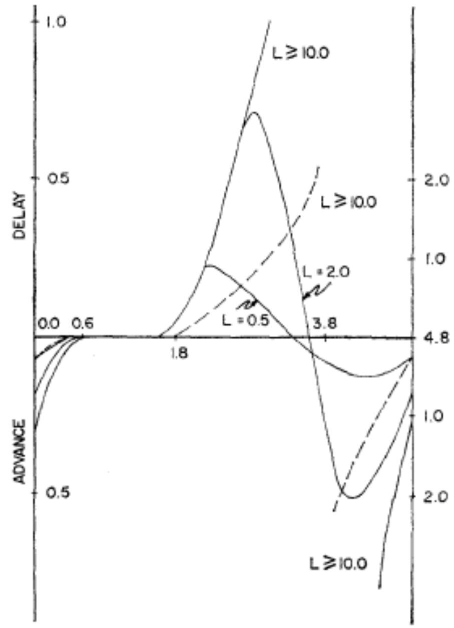



Figure 2. Response curves for light pulses of various intensities $L$ and short duration (0.2). For $L \geq 10$, they are all identical. Because of the much larger phase shifts, such a curve is replotted (broken line) in a smaller scale (shown on the right-hand side)

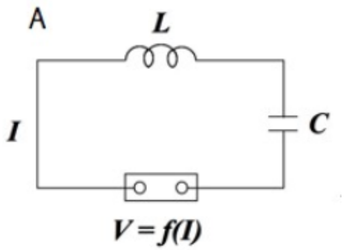

Pavlidis T (1967) A model for circadian clocks. Bull Math Biophys. 29:781-91.
Pavlidis T (1967) A mathematical model for the light affected system in the drosophila eclosion rhythm. Bull Math Biophys. 29:291-310.

# First models for circadian clocks

## Van der Pol Oscillator

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \mu^2\left(y + \left(x - \frac{1}{3}x^3\right)\right)$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -x$$



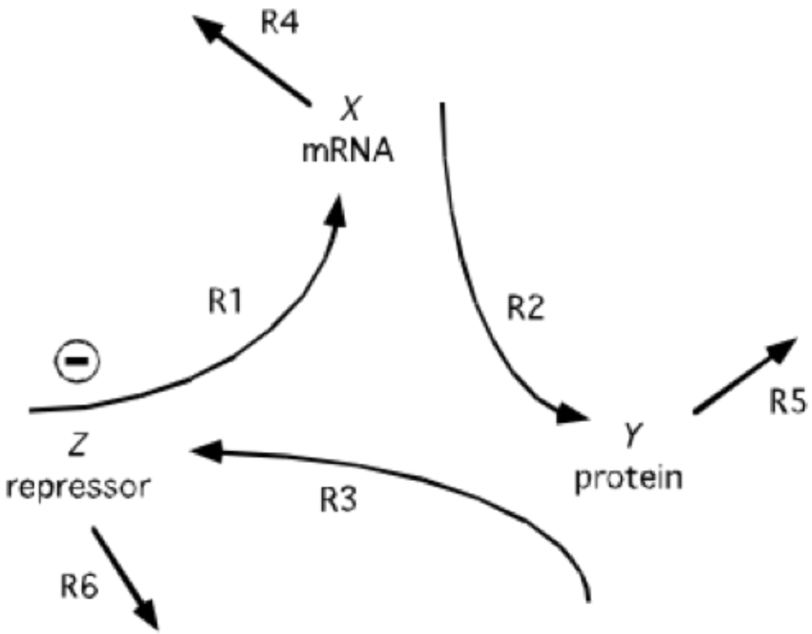

$$\frac{\mathrm{d}s(t)}{\mathrm{d}t} = z(t) + \epsilon\left(\frac{2\pi}{\tau}\right)\left[s(t) - \frac{4s(t)^3}{3\gamma^2}\right] + \left(\frac{2\pi}{\tau}\right)\gamma(1 - ms(t))CI(t)^{1/3} \qquad (3)$$

$$\frac{\mathrm{d}z(t)}{\mathrm{d}t} = -\left(\frac{2\pi}{\tau}\right)^2 s(t) + \frac{2\pi}{\tau} z(t)\left(\frac{1 - ms(t)}{3}\right)CI(t)^{1/3}$$

Kronauer RE, Czeisler CA, Pilato SF, Moore-Ede MC, Weitzman ED. (1982) Mathematical model of the human circadian system with two interacting oscillators. *Am J Physiol*. 242: R3-17.

# First models for circadian clocks

## Goodwin Model (1965)




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

$$\frac{dY}{dt} = k_2 X - k_5 Y$$

$$\frac{dZ}{dt} = k_3 Y - k_6 Z$$

- negative feedback loop
- non-linearity
- delay

Goodwin BC (1965) Oscillatory behavior in enzymatic control processes. **In**: G. Weber, Editor, *Advances in Enzyme Regulation* **3**, Pergamon Press, Oxford, pp. 425-438.

Ruoff P, Vinsjevik M, Monnerjahn C, Rensing L. (1999) The Goodwin oscillator: on the importance of degradation reactions in the circadian clock. J Biol Rhythms. 14:469-79

Ruoff P, Rensing L (1996) The Temperature-Compensated Goodwin Oscillator Simulates Many Circadian Clock Properties. J. Theor. Biol. 179:275- 285.