Biological Clocks

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What is biological clock?

All eukaryotes and some prokaryotes display changes in gene activity, biochemistry, physiology, and behavior that wax and wane through the cycle of days and nights – *circadian rhythm*.

Circadian rhythmicity is universally associated with the ability to perceive light, and the oscillators (“clocks”) giving rise to these rhythms, which are feedback loops based on transcription and translation, are reset by light.

Examples:

- the level of the hormone <u>melatonin</u> that rises in your body during the night and falls during the day.
- fruit flies (**Drosophila**) hatch in greatest numbers just at dawn.

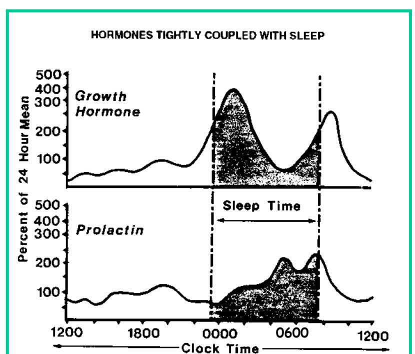
Even when the organism is placed in constant conditions (e.g., continuous darkness), these rhythms persist. However, without environmental cues, they tend to be somewhat longer or somewhat shorter than 24 hours - giving rise to the name **circadian** rhythms (L. *circa* = about; *dies* = day).

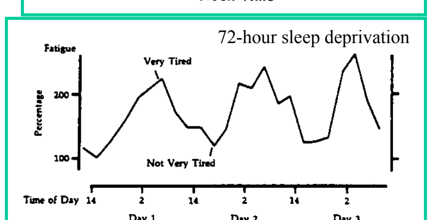
Circadian rhythms

- The most important property is the ability to maintain self-sustained oscillations.
- That is, under constant conditions, the rhythm continues with its natural frequency.
- Can be reset by environmental cues, such as light.

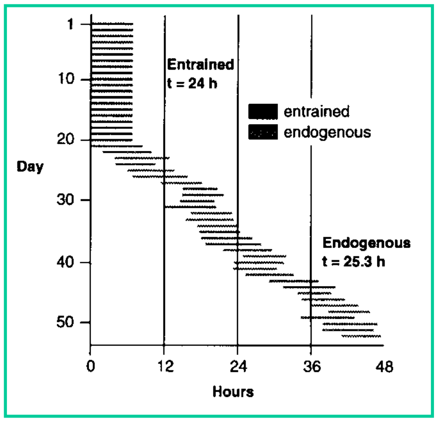
What do circadian rhythms control?

- Wake/sleep
- Hormonal
- Temperature
- Immune
- Drug metabolism
- Renal function

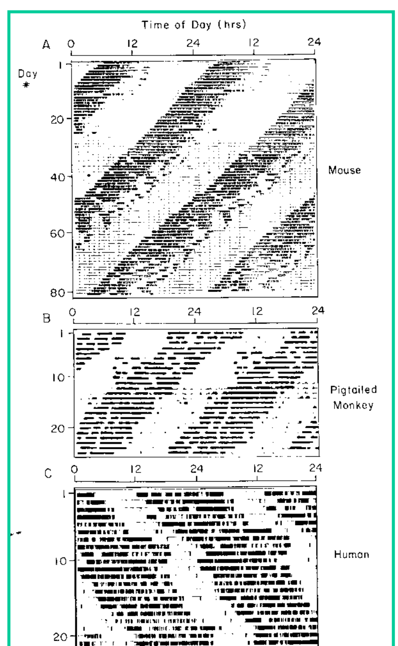




Circadian rhythms -- free run



Circadian Rhythms - Various Species



Circadian rhythms – the pacemaker

In humans and other animals, the suprachiasmatic nucleus (SCN) controls most circadian rhythms such as rest-activity and drinking rhythm.

-- If the SCN is isolated from the rest of the brain by means of knife cuts in vivo, circadian rhythms in single unit activity persist in the resultant "hypothalamic island," while rhythms in unit activity in other brain regions disappear.

-- Lesions of the SCN eliminate circadian rhythmicity in rodents.

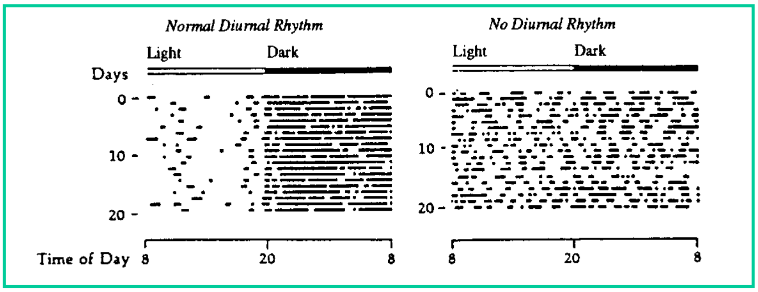
-- Circadian rhythmicity is restored by transplantation of fetal SCN tissue. The newly restored rhythm is that of the donor.

The SCN receives environmental light-dark information from the retino-hypothalamic tract. The cellular pathway by which light entrains the SCN pacemaker is unknown.

Light Entrains the Human Biologic Clock

(Retinohypothalamic tract)

Circadian rhythms after SCN lesion



Melatonin and Circadian Rhythms

- Melatonin is a hormone released from the pineal during darkness.
- It is suppressed by exposure to light.
- In some blinded individuals, it continues to be entrained to the external light/dark cycle.
- Therefore, the retinohypothalamic tract may be intact, transmitting light/dark information to the SCN even though there is no conscious perception of vision.

The Circadian Clock in Drosophila

A number of genes in Drosophila are turned on when the animal is exposed to light:

- •**effector genes** whose products mediate the animal's responses (e.g. hatching or molting)
- •**clock genes** whose products regulate the circadian clock. Three key members of this group are:
  - •***period*** (***per***)
  - •***timeless*** (***tim***)
  - •***vrille (vri)***

Activation of all of these genes requires that their promoters are bound by the protein transcription factors.

- •**CLOCK** encoded by the gene ***clock*** (***clk***) and
- •**CYCLE** encoded by the gene ***cycle*** (***cyc***)

The Circadian Clock in Drosophila – The Mechanism

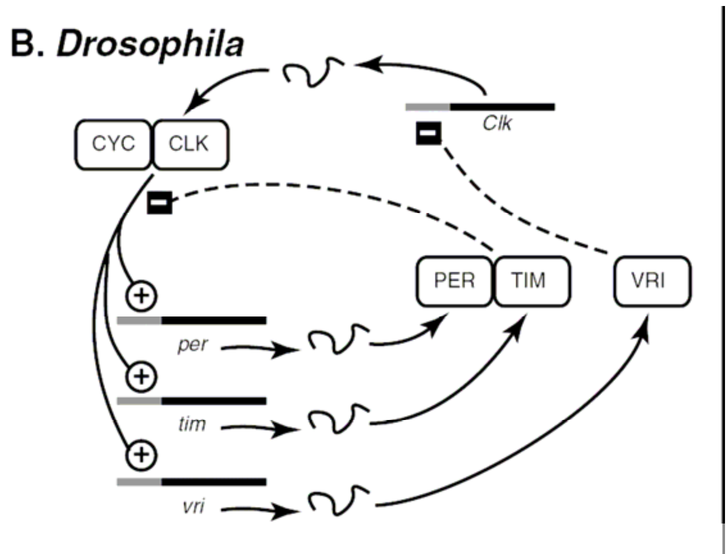
The CLK/CYC proteins are transcription factors for ***per***, ***tim, vri*** and other effector genes.

The **PER** and **TIM** proteins (synthesized on ribosomes in the cytoplasm) form dimers.

- •When the concentration of these gets high enough (early evening), they are transported into the nucleus.
- •The VRI protein also accumulates in the cytoplasm and goes into the nucleus, represses Clk gene transcription.

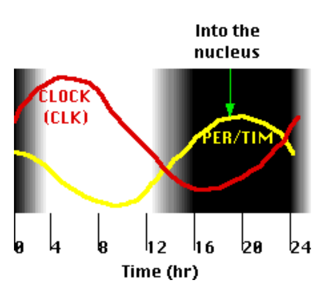
In the nucleus, PER/TIM

1) binds to the **CLK/CYC** transcription factors, removing them from the promoters of the genes they activate; thus shutting off transcription. Because these genes include ***per*** and ***tim***, the result is a negative feedback loop; that is, the products of the ***per*** and ***tim*** genes inhibit their own synthesis.
2) Shuts off ***vri*** transcription, thus remove inhibition on ***clk*** gene transcription.
3) As the level then falls, this inhibition is lifted and PER/TIM activity begins anew.



The Circadian Clock in Drosophila – The Mechanism

•The time required for the different effects results in the levels of **PER/TIM** and **CLOCK** oscillating in opposite phases with a circadian (~24 hr) rhythm.



The Circadian Clock in Drosophila – Setting The Clock

Even without any external cues (e.g., alternating light and dark), the cycles persist although they tend to drift away from environmental time.

- Under natural conditions, the clocks are precise.
- This is because they are "set" (synchronized) by environmental cues, of which light is one of the most important.

In Drosophila, it works like this.

- Light (blue) is absorbed by the protein cryptochrome (**CRY**).
- This causes an allosteric change in its conformation enabling it to bind to **TIM**.
- This causes TIM to break down ending its inhibition on CYC/CLK.
  - If this happens when **PER/TIM levels are rising** (late in the "day"), it sets the clock back.
  - If it happens when **PER/TIM levels are declining** (late in the "night"). it sets the clock ahead.

The Circadian Clock in Mammals

The circadian clock in mammals resembles that in Drosophila in a number of ways with many of the participating genes being homologous. However, there are some differences:

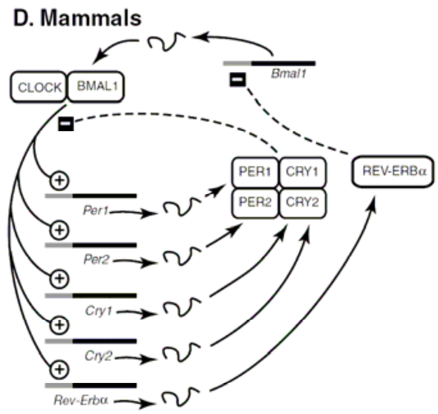
The transcription factors that turn **on** the light-induced promoters are dimers of the **CLOCK** protein and a protein designated **BMAL1**. These dimers turn **on**

- the ***per1 and per2*** genes;
- ***cry1 and cry2*** genes, the gene encoding cryptochrome
- **rev-ERB**α gene, whose product **inhibits** transcription of ***Bmal1***
- effector genes (such as the gene encoding vasopressin)

The **Per** and **Cry** mRNAs are exported to the cytoplasm where they are translated.

- The **PER** and **CRY** proteins then form dimers that enter the nucleus where they
  - turn OFF their own genes (as PER/TIM dimers do in Drosophila);
  - turn OFF ***Rev-erb***α gene, thus removes the inhiibition on *Bmal1* transcription. So this double-negative effect causes the level of the BMAL1 protein to rise.
- These actions cause the levels of BMAL1 and PER/CRY to oscillate in opposite phases (as CLOCK and PER/TIM do in Drosophila).

The Circadian Clock in Mammals



The Circadian Clock in Mammals - Setting The Clock

In mice, light acts through the retina and direct neural pathways to the suprachiasmatic nuclei (SCN) to stimulate per gene expression.

Mice who are totally blind (lacking both rods and cones) have no trouble keeping their circadian clock on time.

They are able to do this because:
-- Some 1 - 2% of the ganglion cells in their retina - instead of depending on signals arriving from rods and/or cones - detect light directly.
-- These ganglion cells have an extensive network of dendrites that contain the pigment **melanopsin**. When exposed to light (diffuse light is fine), these ganglion cells become depolarized and send their signals back to the SCN.

Sleep Disorders

Unlike mice, people who are totally blind cannot set the clock in their SCN. As a result, their circadian rhythm drifts out of phase with the actual cycle of day and night. These people often are bothered by feeling sleepy during the day and wide awake when they want to be asleep at night.

Some people suffer from a disorder called **familial advanced sleep-phase syndrome** (FASPS). As the name suggests,

- it is inherited ("familial") and
- their circadian clocks run fast ("advanced").

Those afflicted tend to wake up several (up to four) hours earlier than normal. **One** cause of the disorder turns out to be a point mutation in the human ***PER2*** gene. As a result, the PER2 protein builds up more rapidly than normal triggering earlier feedback inhibition.

Delayed sleep phase syndrom (DSPS) is the most frequent disorder among young adults. Symptoms such as delayed sleep-wake cycle (4am-noon) are thought to be caused by delayed melatonin production. Genetic cause not entirely clear, may involve PER3.