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Circadian rhythms

How your body clock works

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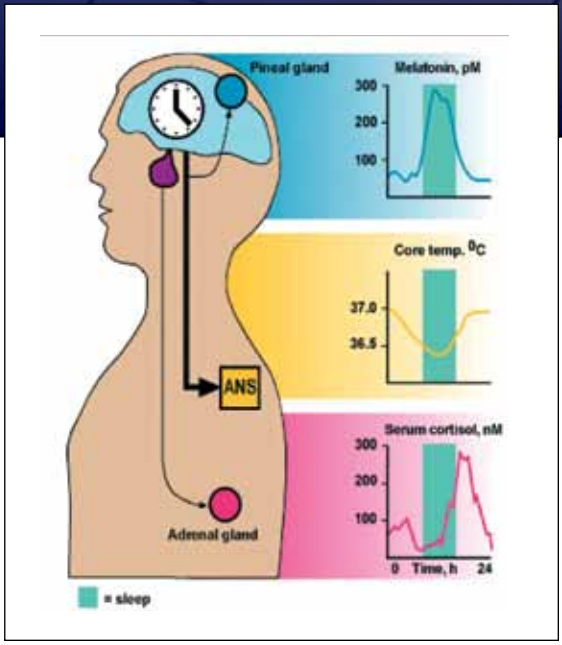
An internal biological clock within every cell of your body helps to co-ordinate and organise human behaviour and metabolism into approximately 24-hour rhythms – allowing organisms to synchronise with, and anticipate, day and night. When the body clock is disrupted in humans it can have serious short- and long-term health consequences, and so understanding how biological time-keeping works has become an important question for medical research. As often happens in science however, the answer just keeps getting more complicated!



How the 'clock' in your brain controls your daily cycle. Rising levels of melatonin bring sleep, during which your core body temperature falls. Then rising cortisol levels wake you up.

We all have body clocks

If you have ever flown across time zones you may have experienced symptoms such as headaches, fatigue, irritability and constipation which are generally referred to as 'jet lag'. We know this is not simply the result of flying abroad, because the same effects are seen in people who do shift work.

What actually happens is that, unlike your wristwatch, the body's master timekeeper (the suprachiasmatic nucleus or SCN, based in the brain's hypothalamus) can only advance or delay by about an hour each day in response to the light levels you experience when awake. So until this clock in the hypothalamus has caught up with the new time zone you're in, it will send muddled hormone signals to other parts of the body. Therefore, for example, even though it might be lunchtime at your destination, your digestive system might think you should be sleeping and is not prepared for food - meaning your meal isn't digested properly.

We know that, although the SCN is very important for co-ordinating rhythms across the body's many organs, in fact every cell and tissue in the body has its own clock – explaining the many observable physiological rhythms such as core body temperature, and the oscillating levels of hormones such as melatonin (that makes us sleepy at night) and cortisol (that wakes us up in the morning).

Disturbingly, there is now a lot of evidence in humans who do shift-work, and from experiments in rodents, to suggest that long-term disruption of the body clock significantly increases the risk of many chronic diseases such as diabetes, cardiovascular disease and various types of neurodegenerative disorder and cancer. This seems to be because the body clock is hard-wired into so many aspects of cellular biology and physiology that when it goes wrong, it impacts upon numerous systems e.g. the body clock controls what time of day cells are able to divide, so when the clock is disrupted, this control is lost and cells become more likely to develop into tumours.

In some countries, you can buy melatonin tablets which may help you get over jet lag; 'anti-energy' drinks contain melatonin and claim to help you relax.

Body clocks = circadian rhythms

'Circadian rhythm' is the term that researchers use to describe this daily biological timekeeping. As with many scientific phrases 'circadian' comes from Latin, meaning 'about daily' (circa–dian). Fascinatingly, circadian rhythms can be observed in most organisms on the planet (including plants, fungi and even some bacteria).

Circadian timekeeping was first observed in 1729 by a Dutch astronomer, Jean Jacques d'Ortous de Marian. He isolated mimosa plants in dark rooms for several days and found that even in the absence of sunlight (or other environmental cues), the plants continued to open their leaves during the day and close them during the night. He concluded that the observed cycle was not a result of external forces but was an innate property of the plant.

Mimosa plants open their leaves during the daytime – even when they are kept in the dark.

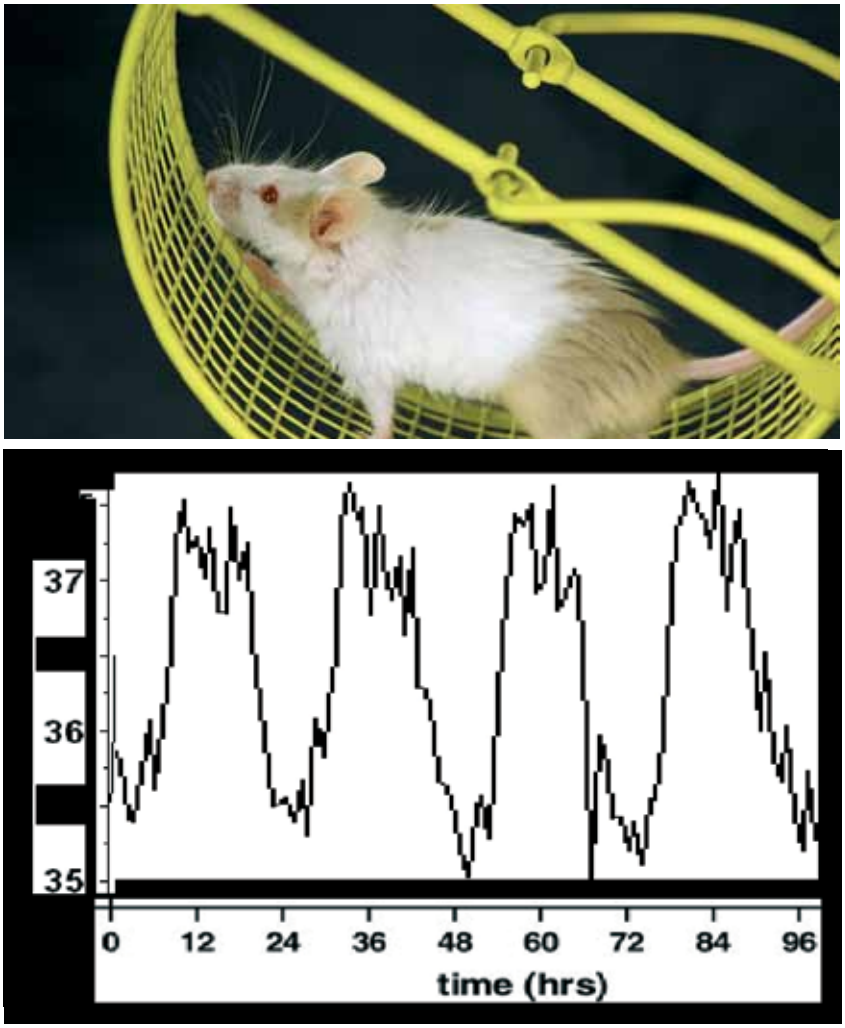
Subsequently behavioural rhythms were observed in humans and many other organisms under similarly constant conditions. Indeed, when measured in the laboratory or underground caves over several weeks, the intrinsic period in humans is slightly longer than a day (~24.2 hours), and slightly shorter in mice (~23.7 hours) meaning that humans would be able to live on Mars without adverse clock impairment (day length of 24.6 hours). In the natural world, the circadian clock is subtly reset each day by cues such as dawn and dusk meaning that the observed period is almost exactly 1 day. It is presumed that the reason for our intrinsic period never being exactly 24 hours, is to provide some flexibility to cope with variable day length over the seasons of the year.

Michel Siffre is a French geologist who has spent long periods underground in experiments to investigate the length of his natural circadian rhythm, which is longer than 24 hours. Sensors on his skin detect his physical condition.

Why be rhythmic?

We think biological rhythms exist because from the beginning of life on Earth (around 3.7 billion years ago) there have always been daily cycles of light and dark (day and night) due to the approximately 24 hours it takes the Earth to rotate on its axis. Therefore organisms which use light from the Sun (directly through photosynthesis, or indirectly due to vision, or for heat) developed the capacity to keep time internally, and thereby anticipate the transitions between day and night. Such organisms would have had an evolutionary advantage over those that did not and so were more likely to reproduce e.g. plants display daily cycles of chlorophyll production which peaks just before dawn, not in response to it, allowing more efficient use of resources. Improved fitness due to internal timekeeping has been confirmed in the lab i.e. plants with an internal clock whose period matches the natural light/dark cycle out-compete plants carrying mutations that result in longer or shorter (28 vs 20 hour) intrinsic periods.

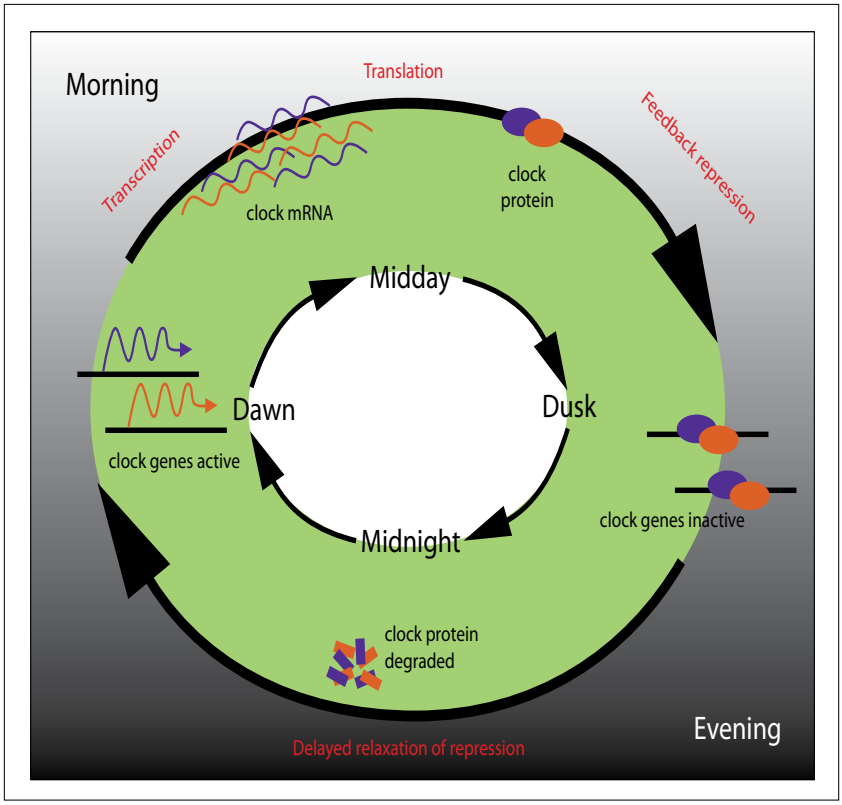
A familiar example of rhythms in humans would be the cycle of sleep and wakefulness which tends to coincide with night and day – this makes sense because humans cannot see well at night. In contrast nocturnal animals like mice, that rely more on smell, do the opposite, sleeping during the day.



Mice show a circadian rhythm in both activity and body temperature.

A genetic basis for circadian clocks

Molecular genetics has been a powerful tool for identifying 'clock genes' which are relevant to circadian rhythms and has taken us several steps towards understanding how organisms keep time. The basic principle is the same whether you are looking at single cells in culture, or whole organisms i.e. when timekeeping is affected by mutating or altering the activity of a gene, then that gene must be involved in clock's mechanism and is labelled a clock gene. For example, a rare human mutation that results in a naturally shorter period (around 23 hours) was shown to be due to a single base pair change in DNA within a gene called Period2, this mutation changes a single amino acid of the encoded protein (PERIOD2) meaning that is broken down more rapidly.



The activity of 'clock genes' varies during the day.

A number of clock genes have now been identified, and the way that they work seems to be quite simple, namely: transcriptional-translational negative feedback loops. Put simply, clock genes in our DNA (within the cell's nucleus) are turned on around dawn and transcribed into messenger RNA (mRNA). The clock gene mRNA is then translated to become clock proteins (in the cell's cytosol), and the levels of clock proteins build up during the day. Around dusk the clock proteins enter the nucleus and turn off their own clock genes. Because there is no longer any transcription (mRNA), no more clock proteins are produced. Overnight, the clock proteins keep clock genes inactive but are slowly broken down so that, by the following dawn, none remain. The clock genes now reactivate and the cycle begins again.

New findings

Although these genetic explanations for circadian rhythms can explain large amount of experimental data, we noticed several clues suggesting that the real mechanism might be more complicated. For example, when other researchers have altered a clock gene's activity so that it is permanently switched on, often the clock does not stop, at the behavioural and/or cellular level. Therefore, to test whether cycles of gene activity in DNA are required for circadian rhythms, we looked in a cell that has no DNA – the red blood cell. Human red blood cells, or erythrocytes, naturally have no nucleus or DNA. We thought that if we could detect circadian rhythms in blood outside of the body, then a transcriptional-translational mechanism could not be the explanation for it.

The author found that even red blood cells, which have no cell nucleus, exhibit circadian rhythms.

To test our hypothesis we looked at rhythms in oxidation of an evolutionarily ancient family of proteins called peroxiredoxins, since we had previously found that these proteins are oxidised with 24-hour cycles in cultured mouse cells and mouse liver and so would be a good 'marker' for rhythms in the absence of gene activity. We therefore took blood samples from three volunteers and purified the erythrocytes, to remove white cells of the immune system, and then incubated the red cells for several days in simple salt/sugar solution.

We observed robust rhythms of peroxiredoxin oxidation that persisted for at least 3 days in vitro (outside the body). Amazingly, we observed similar rhythms in a simple marine alga (called *Ostrecoccus tauri*). This suggests that purely genetic mechanisms are not sufficient to account for cellular timekeeping, and the hunt is now on to determine what additional systems in the cell are able to sustain the remarkable and medically-important phenomenon of circadian rhythms.

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