Circadian clocks - the fall and rise of physiology

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TIMELINE

Circadian clocks — the fall and rise of physiology

Till Roenneberg and Martha Merrow

Abstract | Circadian clocks control the daily life of most light-sensitive organisms — from cyanobacteria to humans. Molecular processes generate cellular rhythmicity, and cellular clocks in animals coordinate rhythms through interaction (known as coupling). This hierarchy of clocks generates a complex, ~24-hour temporal programme that is synchronized with the rotation of the Earth. The circadian system ensures anticipation and adaptation to daily environmental changes, and functions on different levels — from gene expression to behaviour. Circadian research is a remarkable example of interdisciplinarity, unravelling the complex mechanisms that underlie a ubiquitous biological programme. Insights from this research will help to optimize medical diagnostics and therapy, as well as adjust social and biological timing on the individual level.

The daily fall and rise of physiology is so utterly obvious that the underlying biological mechanism remained unexplored for centuries. In synchrony with the fall and rise of the sun, animals sleep and rest, plants open and close their blossoms, and plankton travel up and down the water column. The first indication that daily rhythms were programmed and not just a passive reaction to natural light or temperature cycles came in 1729 from the French astronomer Jean Jacques d'Ortous De Mairan (TIMELINE). He discovered that the mimosa plant continues to fold and unfold its leaflets each day, even in constant darkness[1]. In less than 350 words, De Mairan described leaf movement in general, concluded that it is not controlled by light and darkness, extended his observations to human patients with sleep problems, suggested the investigation of his discovery in other plants (with special consideration of daily temperature cycles), and proposed experiments with shifted light–dark cycles. He apologized for being too busy to do these experiments himself but invited botanists and physicists to pursue his discovery while warning them that progress in true experimental science would be very slow.

Indeed, it took 30 years to show persistence of the daily, rhythmic leaf movement in constant temperature[2,3] and several more decades until physiological experiments proved the endogenous nature of the biological clock in plants[4]. Similar evidence for animals[5] took almost another century and for humans[6] yet another 50 years. In temporal isolation (constant temperature and light (LL) or darkness (DD)), the period of the 'free-running' oscillation often deviates from 24 hours; its exact length depends on conditions (for example, DD or LL), species, and even the individual. The non-exact, circadian (from *circa dies*, about one day) period provided compelling arguments for an endogenous mechanism rather than some unknown exogenous factor, which was connected to the rotation of the Earth, generating daily rhythms. In addition, the free-running period proved to be a heritable trait[7].

By 1960, research on biological clocks had developed into a small new discipline, and 157 pioneers of the young field met for their first international conference in Cold Spring Harbor[8]. In his remarkable and perspicacious contribution, Colin Pittendrigh summarized the qualities of circadian clocks in 16 generalizations[9] (BOX 1). Although most researchers were convinced of the endogenous nature of the biological clock, this was still disputed by Frank Brown[10], who argued that some unknown agent linked to the rotation of the Earth was responsible for the observed rhythms in constant light or darkness. It is noteworthy that the term 'circadian'[11], with which we are so familiar today, was at that time chosen over the designation 'endogenous', partly because it prevented dwelling on the endogenous/exogenous controversy.

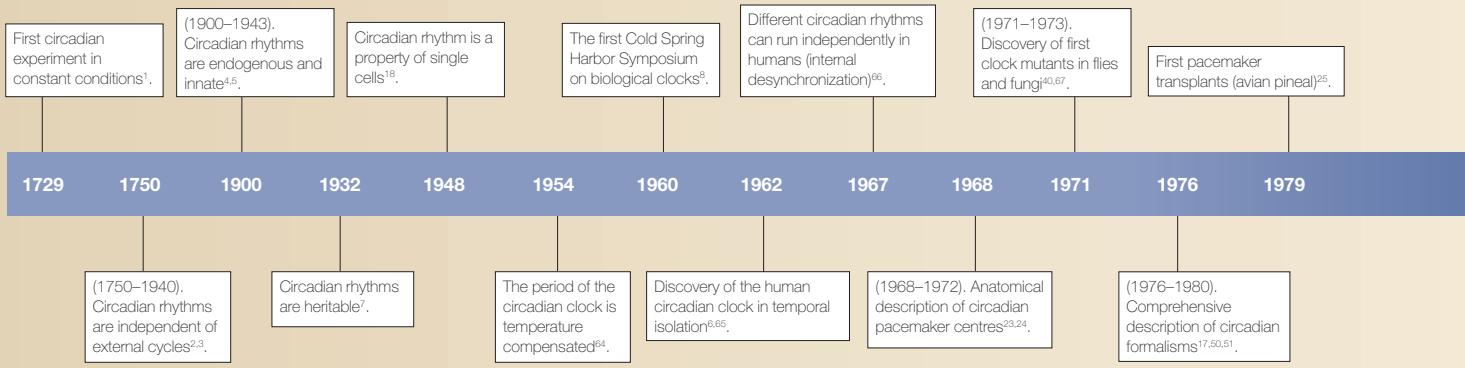
To understand a system, its properties have to be defined, its anatomical localization identified, the underlying mechanisms elucidated, and, eventually, insights have to be put into a broader context. The next sections attempt to summarize these steps for circadian research.

Is the clock a clock?

The '*nomen est omen*' principle has not spared the biological clock. The 'clock' metaphor was frequently evoked up to the twentieth century. Indeed, the system is used as a 'watch' to programme and anticipate the quest for regular food sources or to compute directions by using the position of the sun or stars[12–14].

Ideally, a clock represents the passing of time — 8.64×10^5 milliseconds per day — precisely and reliably. Physical clocks proceed with a constant velocity, independent of other influences, compensating for changes in ambient temperature, spring power or battery charge. Biological clocks are similarly compensated, so that their period is almost independent of temperature[15] (BOX 1, generalization XI) or different metabolic states (BOX 1, generalization XVI). They are also astonishingly precise (BOX 1, generalization VII): for example, Curt Richter recorded the free-running circadian activity–rest cycle of

Timeline | **The landmarks of circadian research***



*The landmarks shown here and described in the main text are highly subjective and — in view of space and reference limitations — cannot give tribute to all the excellent researchers who contributed to the success of this discipline.

a blind monkey for over three years during which its 'free-running' period varied only by a few minutes[16]. Yet, it was not 'on time', in that its period was slightly longer than 24 hours. Circadian clocks become accurately tuned to the 24-hour day if they are synchronized by environmental signals (known as *zeitgebers*; BOX 1, generalization XIII).

Unlike real clocks, the circadian system is highly adaptive and dynamic. Its 'velocity' depends on current conditions (BOX 1, generalizations VIII, XII) and even changes over the course of a cycle[17], representing a biological correlate of the famous melting clocks by Salvador Dali. The behaviour of the clock can also depend on prior conditions (for example, LL, DD or light–dark (LD)) which produce 'after-effects' (BOX 1, generalization X) and, finally, its adjustment to a new time regime can take several days (transients; BOX 1, generalization XV). So, the metaphor 'clock' is not always useful to understand what is a highly adaptive system — a clock for all seasons[18].

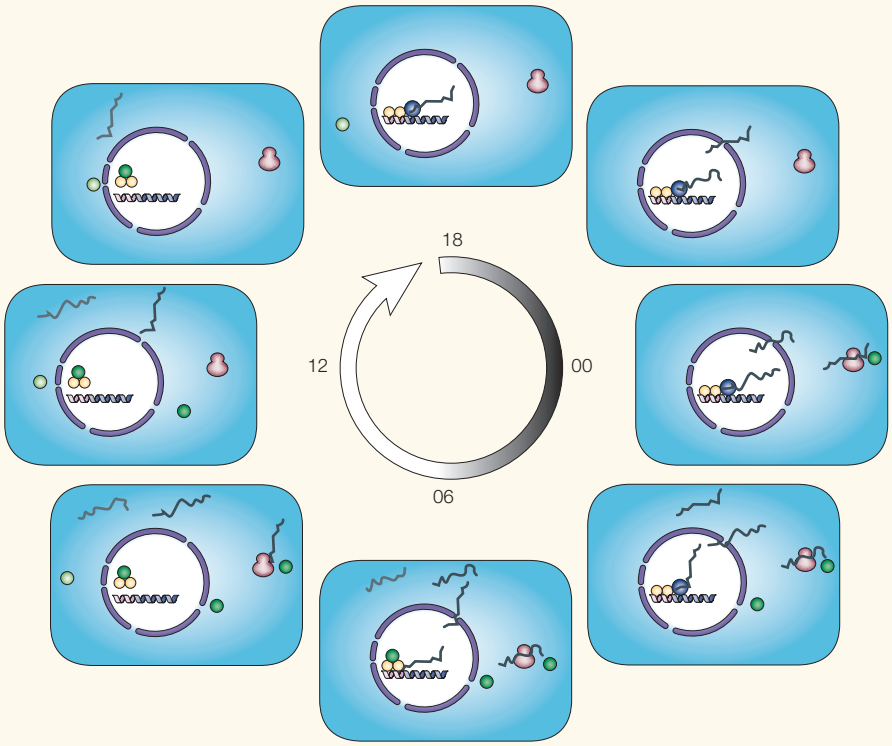


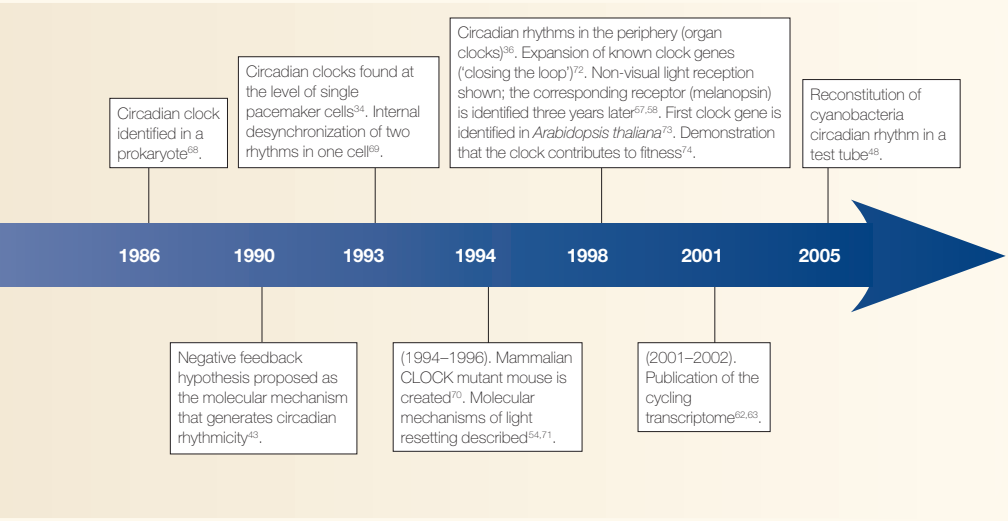
Figure 1 | **The basic concept of the 'transcriptional–translational autoregulatory negative feedback loop'.** This concept is based on the model of Hardin, Hall and Rosbash[43]. Clockwise from the top: a clock gene is transcribed, the resulting RNA (black wiggles) is shuffled to the cytosol and translated into protein (green circles). While more RNA and protein accumulates, clock proteins are translocated back into the nucleus where they inhibit their own transcription. This inhibition persists until the degradation of both RNA (grey wiggles) and protein (light green circles) relieves the inhibition and the cycle restarts. The indicated clock times (in hours) vary between species.

Where is the clock?

By the 1960s, it was clear that a circadian clock was present in practically all eukaryotes, including single-celled organisms, plants and animals. Its absence in prokaryotes became a dogma that was only overthrown in the 1980s by the discovery of a robust circadian clock in photosynthesizing cyanobacteria[19].

Identifying the location of the clock in complex systems became a prime target of circadian research during the 1960s and 1970s. Distinct, dedicated circadian centres (pacemakers) were found in insects (in the optic lobes)[20], molluscs (in the eye)[21] and birds (in the pineal gland)[22]. Ablation experiments and tracing retinal projections by autoradiography eventually led to the exact localization of a mammalian circadian pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus[23,24].

Circadian pacemaker tissues have astonishing properties. When they are ablated, the activity rhythm in constant conditions loses its circadian consolidation and when they are transplanted, they provide the recipient with information for both the period and phase of entrainment of the donor[25,26]. They were the first tissues in which circadian rhythmicity was recorded *in vitro*[27–29]. It was suggested that circadian rhythms are a unique property of the pacemaker tissues, generated by interactions of neurons. This claim was made despite



earlier reports of circadian rhythms in cultured adrenal glands[30] or liver[31], as well as their established existence in unicellular organisms[32]. Cell-autonomous clocks in metazoans were eventually shown for pacemaker cells in the avian pineal gland[33], the molluscan eye[34], the mammalian SCN[35] and, eventually, also for mammalian tissues outside the SCN[36].

The clear demonstration of rhythms in the periphery — organ clocks — was enabled by a luciferase reporter construct that was driven by the promoter of the *mPer1* clock gene. This construct allowed long-term, online measurements of self-sustained rhythms *in vitro*[37]. Yet, even then, the SCN-centric view persisted — SCN rhythms were self-sustained, whereas peripheral rhythms appeared to dampen rapidly[38]. It was later shown that peripheral clocks were also self-sustained by using an alternative clock-regulated promoter (*mPer2*) in the reporter constructs[39]. So, circadian clocks tick in practically all organisms and, within these, in practically all cells.

The molecular clock

Successful genetics requires quantifiable phenotypes, and the circadian clock can be characterized in terms of numbers — for example, the period of the free-running rhythm or when the rhythm occurs during a defined cycle (the 'phase' relationship of the rhythm with respect to the zeitgeber). First indications for the genetic basis of the clock came from experiments with plants[7], but prominent researchers remained doubtful as to whether dedicated 'clock genes' could be defined for a system with such high complexity. Despite overwhelming scepticism, the first clock mutant was found in a mutant screen (which relied on a complex behaviour) using *Drosophila melanogaster*[40], eventually leading to the cloning of the *period (per)* gene[41,42].

At a Gordon Research Conference in 1990, Michael Rosbash and Paul Hardin introduced a hypothesis for how clock genes and their products could generate circadian rhythms[43]. The idea was grounded in the observation that oscillations in the *D. melanogaster per* gene RNA were followed ~4 hours later by mirroring oscillations in the PER protein. As protein levels increased, the RNA would start to recede. The simplest version of the hypothesis describes an autoregulating negative feedback loop based on transcription and translation (FIG. 1): a clock gene is transcribed, its RNA is translated into protein, the protein enters the nucleus, eventually inhibiting its own transcription until degradation of both RNA and protein relieves the inhibition and the cycle restarts.

Over the years, this basic model has rapidly evolved with the discovery of other clock components in flies, as well as in fungi, plants, cyanobacteria and many representatives throughout the animal kingdom. Clock-gene families that were created by gene duplications were found in animals and plants. New mechanisms (for example, positive feedback loops) and additional, coupled feedback loops (for example, see REF. 44) were postulated. Even general cellular regulators, such as protein kinases, have important roles in clock function — mutants of a mammalian casein kinase show one of the most severe clock phenotypes[45].

Clock components can be expressed rhythmically (for example, negative feedback elements, as well as some activators) or constitutively (for example, kinases and other activators). Although the molecular clock appears to use many of the same components across animal species, their role within the loops varies. Whereas cryptochrome is a light receptor in plants, it is a transcriptional regulator in mammals and serves both functions in *D. melanogaster* (for reviews, see REFS 44,46,47). A common molecular theme among clock proteins is the formation of homodimers and heterodimers through PAS domains (homologous regions first identified in *D. melanogaster* Per, Arnt and Sim proteins). This step is important for processes such as nucleo-cytoplasmic translocation and the activation or inhibition of clock-gene expression. Furthermore, because different members of clock-gene families are often

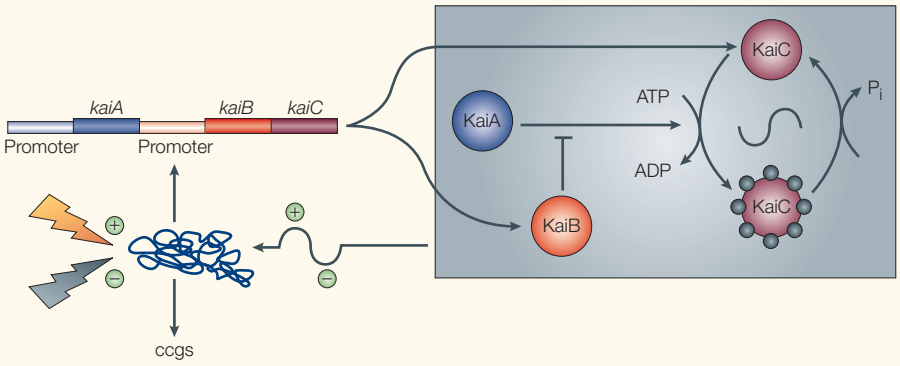


Figure 2 | **Model of the *Synechococcus elongatus* circadian clock.** In this recently proposed model[48], the ~24-hour rhythmicity is generated by a metabolic phosphorylation oscillator, which functions both in light and in darkness (shaded area). KaiA acts positively on KaiC phosphorylation, which can be downregulated by KaiB. This metabolic pacemaker upregulates (+) and downregulates (-) general transcription, transmitting circadian regulation to the cyanobacterial transcriptome (ccgs, clock controlled genes). The transcriptional level of circadian regulation in *Synechococcus elongatus* is therefore an output of the clock. Due to the fact that light upregulates and darkness downregulates transcription, gene expression is also an input to the clock by transiently altering the concentrations of the pacemaker components KaiB and KaiC. Figure modified, with permission, from REF. 49 © (2005) Elsevier Science.

Box 1 | **Empirical generalizations about circadian rhythms**

The generalizations below have been reproduced from REF. 9.

I: ***CR's are defined as those biological rhythms whose τ_{FR} IS AN APPROXIMATION TO THE PERIOD OF THE EARTH'S ROTATION***
This remains the most powerful, though by no means, the only line of evidence justifying III, below.

II: ***CR's are UBIQUITOUS in living systems***
This holds in the systematic sense of kinds of organisms, and the physiological sense of kinds of functions. The emphasis in the literature on rhythms of, say, locomotion and leaf movement reflects only ease of assay for these "superficial" phenomena; rhythms of DNA synthesis, e.g., exist but are less easily followed routinely.

III: ***CR's are ENDOGENOUS in the living system***
This generalization is universally accepted; but one laboratory retains some complex qualifications, and would object to the generalization unless so qualified (See Professor Brown's question in the discussion following this paper.)

IV: ***CR's are usually (if not always) SELF-SUSTAINING OSCILLATIONS***
This is clearly the case in animals; some plant rhythms damp out but it is still not fully clear that this implies real damping of individual cell rhythms or merely their desynchronization which imposes an overt aperiodicity on the whole organism.

V: ***CR's are INNATE***
They are not learned from or impressed by the environment as so much of the older and even comparatively recent literature has suggested. In those systems that are aperiodic if raised from egg or seed in constant conditions, periodicity is elicited by a single (non-periodic) stimulus that in *Drosophila* may be only a 1/2000 sec. flash of light.

VI: ***CR's occur autonomously at both cell and whole-organism LEVELS OF ORGANIZATION***
They have not yet been sought sufficiently at levels lower than the cell for us to know whether they occur (autonomously) even there.

VII: ***τ_{FR} is characterized by a remarkably small variance in a freerunning sequence of cycles; the underlying system displays remarkable PRECISION***
Observed standard errors of the period may be less than 2 minutes per day.

VIII: ***τ_{FR} is not a fixed characteristic of the individual organism; it is open to spontaneous and induced shifts within a range of values***
The limits of this range may (but are not proved to) be characteristic of the individual.

IX: ***Some SPECIES DIFFER clearly from others in the RANGE OF REALIZABLE τ_{FR} values***
There is a suggestion that in nocturnal species the range (measured as τ_{DD} values) is biased below 24 hours; in diurnal species above 24 hours. In some species the range fully spans 24 hours.

X: ***τ_{FR} may show AFTER-EFFECTS of the regime immediately preceding the steady-state freerun being studied***
Evidence for this new generalization is presented later in this paper.

XI: ***τ_{FR} is so slightly temperature-dependent that it is proper to emphasize its near-INDEPENDENCE OF TEMPERATURE***
The known Q_{10}'s range from ~0.9 < ~1.2. This feature suggests the near-independence reflects a *compensation* achieved by a several-component system; and the temperature-compensation is taken by most workers to reflect functional significance of the system as a "clock".

XII: ***τ_{FR} is LIGHT-INTENSITY DEPENDENT***
There is evidence of a fairly strong further generalization which I propose to call *Aschoff's Rule.* This can be summarized by $\tau_{LL} > \tau_{DD}$ in nocturnal animals; $\tau_{LL} < \tau_{DD}$ in diurnal animals.

XIII: ***CR's are ENTRAINABLE by a RESTRCTED CLASS OF ENVIRONMENTAL PERIODICITIES***
Light and temperature cycles are the dominant entraining agents (Aschoff uses the term *zeitgeber*), and in many species probably the only agents. There are many pertinent subsidiary generalizations concerning limits of entrainment (narrower in more complex organisms), etc.. The present writer remains unconvinced by, but must note here, claims of Professor Brown that unknown geophysical cycles are "sensed" by organisms and, in fact, somehow explain the facts summarized by generalizations VII and XI.

XIV: ***The PHASE of a freerunning CR CAN BE SHIFTED BY SINGLE PERTURBATIONS in the light and/or temperature regimes***
The character of the $\Delta\phi$ response is a function not only of intensity and duration of the perturbing signal, but—especially—of the phase at which the CR was perturbed.

XV: ***TRANSIENTS always precede attainment of a new steady-state***
This is true whether the former steady-state was disrupted by a single perturbation or by a $\Delta\varphi$ in the entraining cycle.

XVI: ***CR's have so far proved surprisingly INTRACTABLE TO CHEMICAL PERTURBATION***

highly related, there are numerous combinatorial possibilities for complex formation. Each unique complex could function within a specific feedback loop, and such a network could be the basis for circadian rhythmicity[47].

Whereas, previously, physiological description had been the main tool for the study of circadian clocks, in the molecular and genetic era, investigations into the molecular-loop mechanisms have focused on the processes of transcription and translation. The focus of the field might be about to change again: when the *Synechococcus elongatus* clock proteins KaiA, KaiB and KaiC are incubated in a test tube with ATP, phosphorylation of KaiC oscillates with a ~24-hour period, which is temperature compensated[48] (FIG. 2). Moreover, cyclic KaiC phosphorylation in cyanobacteria persists *in vivo* independent of transcription and translation[48]. Although this could be a unique aspect of prokaryotic clocks, there are numerous indications that rhythm-generating mechanisms that are independent of rhythmic transcription and translation of clock genes exist in eukaryotes[49].

Entrainment

A free-running clock is useful for understanding how a rhythm is generated, but the clock in the real world is practically always exposed to a cyclic environment with entraining zeitgebers (for example, light or temperature). Similar to physical oscillators, circadian clocks respond according to when in the oscillations a stimulus is received. A physically identical stimulus can shift a given phase in the rhythm forward or move it back, or there may be no change at all. These response characteristics have been formally and mathematically described[17,50,51], which is an essential prerequisite for experiments and their interpretation both in the physiological and the molecular eras. These formalisms — common rules for all circadian systems — predict the period length of the free-running rhythm in DD or different intensities of LL, as well as the phase of entrainment in different zeitgebers (photoperiod, stimulus strength or cycle length). They also predict systematic relationships between the free-running period and the phase of entrainment (for a summary of the formalisms of entrainment, see REFS 52,53).

In its most simple form, the circadian system can be conceptualized as having an input pathway, a rhythm generator (oscillator) and an output pathway (FIG. 3). In reality, most circadian systems — at all levels of complexity, including cells — contain several

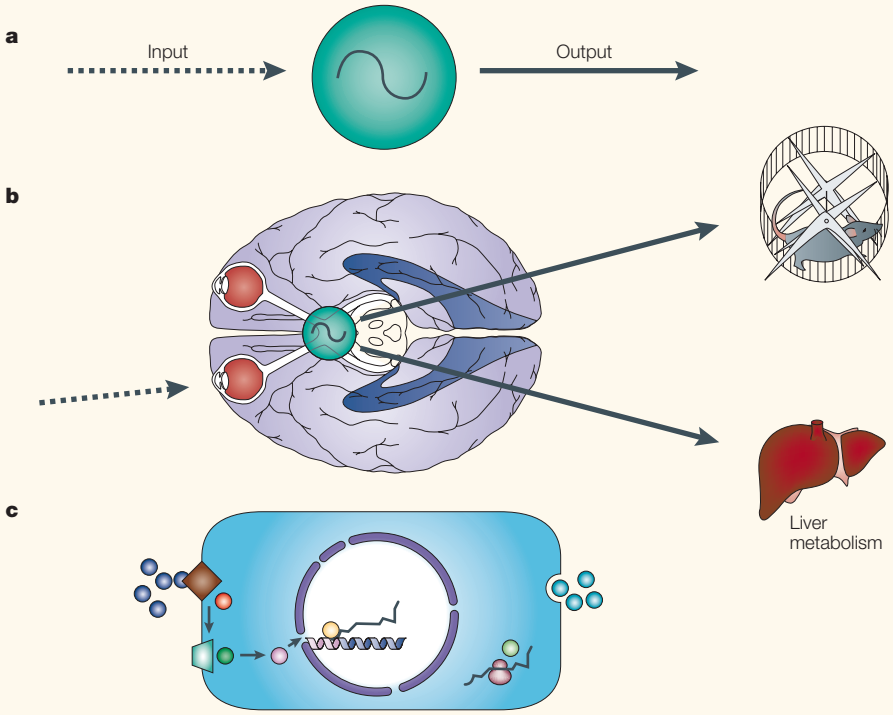


Figure 3 | **Inputs and outputs of the clock. a** | Formally, the circadian system can be depicted as a pathway that comprises inputs (transducing entraining signals), the mechanism that generates the circadian rhythm (oscillator), and outputs. **b** | On the systemic level, the different components can be implemented in different cells or tissues. In the case of mammals, the zeitgeber light is received by receptors in the eye (by rods, cones and melanopsin, which is found in specialized ganglion cells[57,58]) and is transduced via the optic nerve to neurons of the suprachiasmatic nucleus (SCN). The circadian pacemaker in the SCN drives output rhythms as diverse as the activity–rest cycle and the metabolism of the liver and other organs. **c** | Circadian clocks are found in most cells where the same input–oscillator–output pathway applies. Cellular clocks — whether they are a SCN neuron, a pineal or a liver cell — are set by endogenous signals (in some non-mammalian systems even directly by light). By means of a second messenger cascade, the signal eventually leads to a change in the concentration of the products of a clock gene. The molecular clock then regulates the rhythmic expression of genes or other cellular functions, such as the electrical membrane properties or the secretion of factors and hormones.

inputs, several oscillators and many outputs. Circadian clocks regulate many different functions through their multiple outputs; in most organisms — especially plants — the clock receives light through several receptors and input pathways[46]; and, finally, the generation of rhythmicity is often based on the coordination of many cellular oscillators, providing precision and robustness on the tissue level (as in the SCN) or, as described above, by forming oscillator networks on the cellular level[47].

The first insights into how light signals affect (and thereby entrain) the molecular clock came from experiments in *Neurospora crassa*[54], demonstrating acute light induction of a clock gene. Further insights[55] clearly demonstrated that — similar to phase shifting, which is not uniformly induced at all times of day — some clock genes are gated for their light induction. Transcription of *mPER2* is robustly induced following a light pulse in the early night, but not in the late night. An alternative mechanism for light reaching the clock is degradation rather than induction, as is the case for the *D. melanogaster* clock protein TIMELESS[44]. Based on the transcription–translation loop concept, the induced changes will alter the phase of the molecular rhythm, thereby adjusting the biological clock to the external environment. Recent results show that transcription and translation can be dissociated in different photoperiods, indicating that entrainment at the molecular level might be more complex[56].

The transduction pathways are also drastically different between organisms: light can directly affect a clock component or, as in mammals, it can be received by retinal photoreceptors, which transduce the signal via transmitters to SCN neurons (FIG. 3). The dissection of the light transduction pathway to the mammalian pacemaker, SCN, is an especially exciting chapter in circadian research. Light entrainment of the mammalian clock is achieved exclusively through the eyes. Although the retina was thought to be completely understood for 150 years, light still perfectly entrained the clock in mice with no rods and cones[57]. This stunning result led to the discovery of an entirely new (non-visual) light transduction pathway and, eventually, to the description of a new retinal, opsin-based photoreceptor, melanopsin[58].

The clock in real life

Physiological approaches, which fell behind during the search for molecular 'ticking' mechanisms, are now on the rise again, as we try to understand what can go awry with timing in humans — with important consequences for health and society. Circadian formalisms are not only useful for probing the clock in experimental conditions, but also for understanding its function in real life. The duration of the zeitgeber light (photoperiod) can drastically change with season, and some individuals respond with winter depression, which can be effectively treated by light. The amount of daily light exposure differs greatly between farmers and office workers, with unknown long-term consequences — for example, for the quality of sleep. These factors, together with an individual's endogenous free-running period, all have an impact on the phase of entrainment in experimental animals as well as in humans. They determine how our individual daily life is organized (chronotypes): some people fall asleep at around 8 pm and wake up spontaneously at 4 am, just when the late chronotypes are going to bed! 'Larks' and 'owls' represent the extremes of a broad chronotype distribution, and the importance of these genetic traits[59] and the factors that modulate them (such as light and age[60]) are only now beginning to be investigated. A detailed knowledge of the individual expression of the circadian clock as it determines and shapes chronotype is relevant for adequate cognitive function, mood and performance — and for determining school times and shift-workers' schedules. Among many other practical applications, circadian research also provides recommendations for administering drugs to attain maximum efficacy with lower doses and fewer side effects.

The mammalian clock is highly complex with specific timing at the level of organs and cells. With modern techniques, these multiple temporal programmes can be separately monitored, and we find that they can be uncoupled under certain conditions and

might even respond to different zeitgebers, such as light for the SCN and food for the liver[61]. With the help of gene arrays, we can monitor which genes are clock regulated[62,63], whether they are key enzymes of metabolic pathways or components of the cell cycle. Approximately 3% of the transcriptome is expressed in a circadian manner in each tissue, but there is little overlap between tissues (except for many of the known 'clock' genes). Taken together, circadian regulation concerns a large proportion of the genome.

Gene arrays only disclose a limited set of regulatory processes, but circadian regulation also involves many other levels — translation, protein modification, degradation and possibly even pH and calcium levels. The predominantly physiological research of decades ago has paved the way for excellent molecular research that has advanced our understanding of molecular clock mechanisms. The molecular insights now have to be put back into the context of physiology to allow an effective application of circadian insights in real life, ranging from understanding the temporal aspects of ecology, the impact of artificial lighting and heating on human clock function, improving medical diagnosis and treatment to decreasing the risks of shift work by developing more biologically adapted social schedules.

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OPINION

Radical medicine: treating ageing to cure disease

Toren Finkel

Abstract | The incidence of many diseases rises sharply with age. Although clearly separable, ageing and certain age-related diseases might share common mechanisms. Cellular metabolism and subsequent generation of reactive oxygen species might contribute both to the rate at which we age and to our susceptibility to numerous chronic diseases, therefore therapies that directly target the ageing process might provide new ways to treat human diseases.

Physicians and scientists looking for insights into disease mechanisms often turn to epidemiologists for clues. Clinical epidemiologists can provide useful associations such as those between smoking and lung cancer, hypertension and cardiovascular disease or, more mundanely, the unfortunate food poisoning incident at the church picnic and Aunt Nellie's potato salad. It is therefore curious that for a wide range of diseases the biggest epidemiological clue has for many years been largely ignored. A stroll down any hospital ward will reveal that although the diagnosis varies from bed to bed, one attribute is common to most patients — they tend to be elderly.

Age represents the greatest risk factor for many diseases. For example, the chance of getting cancer if you are over 65 is 10 times greater than if you are under 65. Similar relationships hold for both neurodegenerative and cardiovascular diseases (FIG. 1). What is this epidemiological statistic trying to tell us? Why does the incidence of certain diseases seem to rise exponentially rather than linearly with age? Although there are several possibilities, I believe that organismal ageing and certain age-related diseases share common underlying mechanisms. Indeed, a growing body of evidence indicates that reactive oxygen species (ROS), which are primarily generated by mitochondrial metabolism, can fuel both processes. This commonality indicates that although disease-specific approaches, such as the use of chemotherapy to treat cancer or the drug L-dopa (L-3,4-dihydroxyphenylalanine) to treat Parkinson's disease, might effectively relieve symptoms, strategies that directly target the ageing process might ultimately provide a new class of therapies to treat a wide range of chronic conditions. Although such strategies are still in their infancy, it is probable that anti-ageing medicines will be most useful in delaying the onset of symptoms and in slowing disease progression rather then reversing previous damage. Here, I review the evidence that ROS contribute to neurodegenerative disease, atherosclerosis and cancer and then discuss how these observations imply a role for anti-ageing strategies for the treatment of these and other human diseases.

Oxidents and neurodegeneration

Amyotrophic lateral sclerosis (ALS) is a devastating disease that is characterized by motor-neuron degeneration, which leads to progressive muscle wasting. Approximately 10% of cases are inherited, and roughly a quarter of these inherited forms result from mutations in the cytosolic form of the antioxidant protein superoxide dismutase (SOD1)[1]. This discovery over a decade ago provided one of the most tangible clues linking the inappropriate metabolism of cellular ROS to the development of a specific neurological disease. That said, how disease-causing mutations in *SOD1* lead to ALS remains controversial[2,3].

A link between oxidative stress and neurological disease also extends to Parkinson's disease, another progressive age-related disease that slowly destroys a selective region of the brain stem. Similar to ALS, most cases are sporadic, although a number of dominant and recessive inherited forms have been described. At least five separate genes that are associated with Parkinson's disease have been identified, including those encoding α-synuclein, parkin, ubiquitin C-terminal hydrolase-1, DJ1 and PTEN-induced kinase-1 (PINK1) (REFS 2,4). How these genetic determinants cause disease remains unclear, although one attractive unifying hypothesis is that they trigger an increase in neuronal ROS levels. Evidence to support this comes from several independent lines of investigation. For instance, in the brains of patients with Parkinson's disease, α-synuclein is modified by oxidative and nitrative stress[2–4]. Inhibiting mitochondrial function leads to increased α-synuclein aggregation, which can, in turn, lead to impaired mitochondrial function[5,6]. Similarly, in both *Drosophila melanogaster* and mouse models of parkin deficiency, there is specific evidence for mitochondrial defects[7,8]. In addition, neurons that lack DJ1 were recently shown to have an increased sensitivity to oxidative stress[9,10]. Finally, PINK1 has a mitochondrial-targeting sequence and in this subcellular location, it seems to function as a kinase that is required for mitochondrial function[11]. These molecular observations come in the context of clinical studies that indicate that mitochondria from patients with Parkinson's disease have reduced function and that chemical inhibitors of the activity of mitochondrial complex I induce a