Introduction to Chronobiology

Chronobiology is a multidisciplinary branch of science dealing with study of biological rhythms. The free-running biological rhythms reflect the endogenous mechanisms of cyclic temporization whose expression is morphologically seen as an internal clock called body clock. All levels of biological integration ranging from ecosystem to subcellular structures exhibit rhythms with diverse frequencies. Periods of most of the documented biological rhythms match with that of any one of geophysical cycles present in the nature. Though circadian rhythms are the most prominent one, ultradian, infradian and circannual rhythms also play vital role in chronobiological homeostasis.

Circadian rhythms are generated by an internal clock, or pacemaker. Therefore, even in the absence of cues indicating the time or length of day, circadian rhythms persist. Circadian rhythms exist even in single cells. In fact, studies have shown that a wide range of cell functions exhibit circadian rhythms. Specific genes called clock genes code for circadian rhythms. Genetic control of circadian rhythms has been examined most extensively in the fruit fly. In mammals, considerable experimental evidence indicates that a region of the brain called the suprachiasmatic nucleus (SCN) is the circadian pacemaker. The SCN, composed of a cluster of thousands of small nerve cells, is located within a region of the brain, called hypothalamus.

Annual biological cycles are widespread in regions both at extreme latitudes as well as close to equator. Activities such as reproduction, growth, molt, migration, and hibernation are timed in an adaptive manner. The most widely used environmental cue for predicting favorable timing of breeding is photoperiod or day length. Photoperiod is the length of the light phase in each daily (24 h) light–dark cycle. Photoperiodism is the biological process of responding to changes in photoperiod.

Chronobiology research requires specialized techniques as well as specialized sets of properties in the model systems. Historically endogenous rhythmicity was first observed in plant leaf movement. Regulation of photosynthetic machinery was studied in primitive cyanobacteria. Invertebrate groups studied extensively for circadian functional organization. Structural diversity of circadian system was studied in non-mammalian vertebrates. Preferred means of studying human chronobiology is autorhythmometry or self-measurement.

Procedures for the analysis of circadian rhythms are part of the broader set of procedures involved in time series analysis in general. Chronobiology has been oriented mainly towards the methods of periodic regression analysis. How one chooses which test of rhythmicity to use is a complex process. Regardless of the test used, once an investigator has determined that a data set exhibits circadian rhythmicity, one should determine the characteristics of the rhythm.

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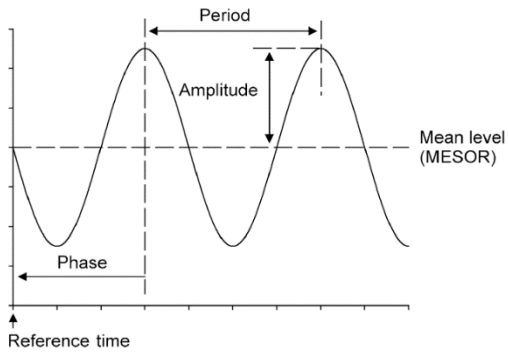
Representation of a typical rhythm



Ideal rhythmic processes, such as those commonly analysed by engineers, are fully characterized by four parameters: MESOR (or mean level), period, amplitude, and phase. The full characterization of biological time series requires two additional elements *viz.* waveform and prominence. Waveform refers simply to the shape of the wave. The prominence of a rhythm refers to its strength and endurance. It corresponds to the proportion of the overall variance accounted for by the signal. It is thus a measure of the signal-to-noise ratio.

Social opportunities and work demands have caused humans to become increasingly active during the late evening hours, leading to a shift from the predominantly diurnal lifestyle of our ancestors to a more nocturnal one. This voluntarily decision to stay awake long into the evening hours leads to circadian disruption at the system, tissue, and cellular levels. These derangements are in turn associated with clinical impairments in metabolic processes and physiology. Unlike other animals, humans are unique in that they often voluntarily shift their activity period to an abnormal time of day, effectively forcing a misalignment between their activity period and their internal circadian clock.

The objective estimation of human circadian phases would be useful in the prevention of circadian rhythm-related diseases. Chronotherapy requires convenient methods for monitoring human circadian phases to ensure effective chronomedicine or circadian medicine. We propose providing non-pharmacological 'circadian therapy' for leading healthy life as follows:

Chronotype assessment – To understand preferred activity schedule without any social compulsions.

Sleep diary assessment – To calculate sleep debt based on sleep pattern for at least 10 consecutive days.

Prakriti type assessment – To correlate chronotype and prakriti type for an Ayurvedic perspective of chronobiology.

Nasal cycle assessment – To analyse rhythmic pattern of breathing as an ultradian rhythm, correlating pulse diagnosis (*nadi pariksha*) with nasal cycle.

Lifestyle management workshops for holistic circadian lifestyle – To provide customize remedies based on above assessment with respect to circadian activity-circadian diet-circadian light.

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Glossary

Actogram: A graphical representation of an organism's phases of activity and rest over the course of a day.

Amplitude: The extent of an oscillatory movement, measured from mean to extreme value.

Chronobiology: The study, at all levels of organization, of adaptations evolved by living organisms to cope with regularly occurring environmental cycles.

Circadian rhythm: A biological rhythm that persists under conditions of constant environmental factors with a period length of about a day, whose phase can be reset by a brief interruption in the constant regimen, and whose period length is relatively independent of temperature within the physiological range of normal growth.

Circadian time (CT): Subjective internal organism time in which one circadian period is divided into 24 equal parts, each a circadian hour. By convention, CT0 corresponds to subjective dawn and CT12 to subjective dusk.

Circalunar: Having a period length of about a month.

Circannual: Having a period length of about a year.

Circatidal: Having a period length of about one tidal cycle, usually 12.4 hrs.

Clock controlled gene (ccg): A gene whose expression is rhythmically regulated by a clock.

Diurnal: Referring to a rhythmic behaviour or process that peaks in the day time rather than at night.

Entrainment: The process by which an environmental rhythm such as the day-night cycle regulates the period and phase relationship of a self-sustained biological pacemaker.

Forced desynchronization: The process in which two mutually entrained oscillators assume different period lengths and move out of entrainment as a result of exposure to an entrainment regimen.

Free run: The state of an oscillator when not influenced by any external time cues.

Free running period (FRP): The period length of a biological oscillator, also called *tau*.

Infradian: Having a period length of greater than one day.

Masking: The phenomenon in which the external factor interferes with the expression of a rhythm or with observation of the behaviour of the pacemaker by directly affecting expression of the overt rhythm.

Melatonin: N-Acetyl-5-methoxytryptamine or 5-hydroxytryptamine is a hormone produced by the pineal gland that contributes to entrainment of the circadian clock in mammals.

Oscillator: It is a set of components within a cell, whose action and regulatory interaction are sufficient to produce a rhythm.

Overt rhythm: A rhythm is an observable characteristic that is directly or indirectly linked to and controlled by the actual pacemaker.

Pacemaker: A localizable, functional anatomical region capable of both sustaining its own oscillations and of entraining other oscillations.

Period: The time after which a defined phase of an oscillation recurs.

Phase: The instantaneous state of an oscillation within a period.

Phase angle(Ψ): The difference between an identifiable phase in one oscillation and the corresponding phase point in another oscillation, such as the difference expressed in hours or in degrees of arc between the peak in a driving oscillator and the peak in a driven or entrained oscillator.

Phase Response Curve: A map of phase-dependent resetting that is, the phase-dependent response of a circadian clock to an entraining agent delivered at different times through a circadian day.

Phase shift(ϕ): The steady state change in phase brought about by the action of an entraining agent.

Photoentrainment: Entrainment brought about by the action of a light cycle.

Photoperiod: The time of light in a light-dark cycle.

Photoperiodic time measurement: The detection of changes in day length by living organisms.

Photoperiodism: The use of changes in the day length on an annual basis, and to regulate seasonal behavioural or physiological processes.

Rhythm: A non-random series of events without any statement of causation.

SCN: The suprachiasmatic nucleus of the ventral hypothalamus; the chief mammalian circadian pacemaker; the master clock.

Spontaneous desynchronization: The phenomenon in which two oscillators that had been mutually entrained spontaneously move out of phase with one another.

Subjective day (Photophil): The portion of a circadian day in a constant darkness corresponding to the day phase in a light-dark cycle.

Subjective night (Scotophil): The portion of a circadian day in constant darkness corresponding to the night phase in a light-dark cycle.

Synchronizer: An agent that promotes synchrony between or among oscillators.

Synchrony: The state in which two or more oscillators are oscillating in phase with the same period length.

Temperature compensation: Ability to maintain intrinsic period, phase, and amplitude of the rhythm despite external fluctuations in temperature.

Ultradian rhythm: Having a period length of less than one day.

Zeitgeber: Environmental signal that can phase-set circadian clocks.

Zeitgebar time: Since strong Zeitgeber defines the rhythm of the clockwork, time is expressed as Zeitgeber time (ZT). ZT0 is defined as “lights on,” the beginning of the light phase, and ZT12 corresponds to “lights off,” the end of the light phase.

To reiterate, the experimental manipulations that chronobiologists impose lead to two new ways of talking about time. When an organism is free-running, we speak of circadian time (CT). When an organism is entrained to an artificially-imposed, environmental daily cycle, we speak of *zeitgeber* time (ZT)**.**

Circadian Clocks as Adaptations

Conditioning / adaptive evolution due to geophysical cycles

The Earth has the unique geophysical condition where environmental factors such as light and temperature change cyclically with a period of approximately 24 h. Through long-term adaption to this cyclic environment, organisms have evolved endogenous and self-sustained timing-keeping mechanisms, namely, the circadian clock. The circadian clock modulates almost all fundamental life processes from molecular, biochemical, cellular, physiological, to behavioral levels and allows for organisms to anticipate environmental changes and coordinate physiological and metabolic homeostasis. In mammals, the core oscillator located in the suprachiasmatic nucleus (SCN) of the hypothalamus drives circadian rhythms, such as hormonal production and activities of neural circuits [1]. In nonmammalian vertebrates including zebrafish, the pineal gland is sensitive to light, secretes melatonin rhythmically, and regulates the circadian system.

One may refer to it as either a continuous process by which organisms adapt to a given environment, or as a character/trait that confers higher fitness to organisms in a given scenario.

Adaptive significance of biological clock

Intrinsic advantage - circadian clocks evolved to ensure temporal segregation of cellular and physiological processes within organisms. Since the efficient operation of multiple processes at the same time would require large energy expenditure, timing them with appropriate temporal lag may help efficiently partition resource/energy.

Extrinsic advantage - some of the environmental factors may be beneficial to organisms, others may be detrimental. circadian clocks may confer adaptive advantages by facilitating entrainment and appropriately timing behaviours so as to avoid harsh environmental conditions, enhance food and mate procurement and facilitate predator evasion thereby establishing a temporal niche to reduce interspecific competition.

Pittendrigh proposed that organisms might have evolved timekeeping mechanisms to anticipate and escape from such harmful effects of light, referred to as 'escape from light' hypothesis.

First proposed by Levandowsky and later elaborated by Kippert, the endosymbiotic coordination theory suggests that since evolution of eukaryotes was facilitated by endosymbiosis of prokaryotes that formed the precursors for currently observed organelles in eukaryotes, such spatial compartmentation of autonomously functioning organelles might have required a coordination mechanism to temporally regulate processes among themselves which otherwise would lead to chaotic cellular system.

If synchrony with the external environment does confer any fitness advantages, then it can be hypothesised that circadian clocks might have evolved to facilitate the synchrony between organisms and the external environment. This forms the basis of the 'circadian resonance' hypothesis according to which organisms are expected to perform their best (enhanced physiological efficiency) when their clock period matches with that of the environmental cycle.

Circadian clocks may continue to persist even under arrhythmic environmental conditions by virtue of the intrinsic advantages conferred by them.

It is reasonable to infer that circadian clocks by virtue of genetic correlations with life-history traits confer adaptive advantages to the organisms by appropriately timing rhythmic behaviours so as to enhance the organism's fitness in a given environment.

Suprachaismatic nucleus (SCN) as a mammalian pacemaker

Light is detected exclusively by the retina, in large part by intrinsically photosensitive retinal ganglion cells (ipRGCs) which express the non-visual opsin, melanopsin. Neural signals from these cells are conveyed to the SCN via the retino-hypothalamic tract (RHT). Thereby, the phase of the SCN clock is indirectly reset in response to light, and in turn, timing information is relayed to the network of peripheral clocks via a complex combination of blood-borne signals, feeding-fasting rhythms and core body temperature changes.

Within the same genus, the circadian system is significantly more sensitive to light in shade-dwelling species than in those species adapted to live in more brightly illuminated areas.

Cytologically, the SCN contain both neurons and astroglia with an estimated ratio of 7–8:1 in the rat SCN. The SCN are bilobed, situated on either side of the ventral floor of the third ventricle in the periventricular zone of the anterior hypothalamus. In the adult laboratory rat, they are ~0.7 to 1 mm in length. Physiologically, four key features define circadian timekeeping in the nocturnal rodent SCN: (1) The SCN exhibits daily changes in the uptake of 2-deoxyglucose, a marker of metabolic activity. (2) electrophysiological recordings show that SCN neurons of nocturnal rodents are spontaneously active and intrinsically generate ~24 h rhythms in the frequency of action potential (AP) discharge. (3) The 24 h variation in electrical activity does not depend on 'network' properties as dissociated SCN neurons isolated in culture also vary daily discharge of AP firing. (4) SCN neuronal clocks are predisposed to synchronise their activity with another, and intercellular communication is necessary for this process.

SCN input – 1) The retinohypothalamic tract (RHT) is a monosynaptic pathway from melanopsin-containing retinal ganglion cells to the SCN,

2) The geniculohypothalamic tract (GHT) mostly innervates the ventral and central aspects of the rodent SCN and originates from neurons in the intergeniculate leaflet (IGL) of the visual thalamus,

3) The median raphe (MR) innervates the ventral and central SCN aspects, and the neurotransmitter serotonin (5-hydroxytryptophan or 5-HT) is the characteristic neurochemical of this pathway.

In the SCN, the terminations of the RHT, GHT, and MR pathways overlap, particularly in the ventral aspects. Perhaps unsurprisingly, activation of non-photic pathways can limit the resetting effects of light pulses, while acute light exposure can reduce or eliminate shifts to non-photic stimuli. Thus, SCN neurons actively integrate photic and non-photic cues to shape the phase of the molecular clock and the entrainment of the circadian system to the external world.

Visualisation of gene expression by in situ hybridisation indicates that not all regions of the SCN rhythmically express clock genes at the same phase or perhaps at all. While the SCN as a whole functions as the mammalian brain's master circadian clock, intra-SCN timekeeping is heterogeneous with some areas appearing to lead daily changes in molecular clock activity, while others follow.

Neurochemically, all SCN neurons contain GABA, but they can, to an extent, be distinguished by the neuropeptides that they synthesise. The prominent neuropeptides contained in SCN neurons include vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), and arginine vasopressin (AVP).

The peptide prokineticin-2 (PK2) is synthesised in the mouse SCN and is implicated in conveying circadian information to the rest of the brain. Levels of PK2 mRNA in the SCN vary across the light-dark and circadian cycles.

Most of our current knowledge of the biological timekeeping mechanisms in mammals arises from laboratory investigations focused on nocturnal rodent models, but studies in diurnal species are much more limited. Comparative analysis of diurnal species from different taxonomic groups is necessary to identify convergent adaptations that are common to a diurnal niche and therefore more likely to be shared by most diurnal species, including humans.

A fundamental property of the circadian system is the PRC which describes the resetting effects of light on the SCN clock. As stated earlier, the shifting effects of light on the SCN clock depend on the time of day when light is applied. With pulses of light given during the night, the pattern of PRC appears to be quite similar across a wide range of diurnal and nocturnal species.

The typical organisation of the SCN into 'core' and 'shell' described in nocturnal species seems to be present in some but not all diurnal species.

Patterns of Per1 and Per2 expression, with high levels during the light phase and low levels at night, have been found in all diurnal rodent species studied so far.

Summary and Questions of Interests

- SCN neurons exhibit intrinsic circadian variation in molecular, metabolic, and electrophysiological characteristics.
- Regional differences in neurochemical and timekeeping characteristics in the SCN are pronounced in some species.
- SCN molecular clock does not appear to differ between nocturnal and diurnal species.
- What processes and mechanisms make an animal diurnal?
- How do SCN output signals influence activity in specific target areas?
- Why are 'core' and 'shell' compartments more discernable in some species and not others?
- What are the mechanisms underlying temporal niche switching within the same species?

Circadian phase markers

Unlike nonhuman models, scientists do not have direct access to the SCN in humans and instead use marker rhythms driven by the SCN to indicate phase, amplitude, and period of the circadian clock.

The most commonly used circadian marker rhythm in humans is the melatonin rhythm. Melatonin is easily measured in saliva, blood, and urine. Two other commonly used circadian marker rhythms in humans are body temperature and cortisol.

Accurate assessment of circadian period in sighted humans requires assessment in the absence of external synchronizers, or under tightly controlled exposure to synchronizers, which ensures their even distribution with respect to circadian phase.

The phase of the melatonin rhythm can also be assessed without performing a constant routine. As noted, melatonin, as opposed to temperature and cortisol, is less impacted by posture and meals. As long as light is maintained at dim levels and food and posture are controlled prior to sample collection (e.g., food proscribed 30 min and posture consistent 15 min immediately prior to the sample), saliva and blood samples can be used to accurately assess melatonin levels. Generally, the dim-light melatonin onset (DLMO) can be determined from samples obtained starting ~7 h prior to and ending ~1 to 2 h after habitual bedtime, assuming that the subject is stably entrained.

Frequently sampled melatonin, temperature, and cortisol data from forced desynchrony protocols are often analyzed with harmonic regression models using an exact maximum likelihood fitting procedure. Such techniques utilize the frequently sampled data available in the dataset and are thus robust and provide the most precise estimates of circadian period and amplitude. Alternatives include fitting linear regression through daily circadian phase estimates, which are less precise as they include error in the phase estimate as well as show higher variance in the period estimate.

Other circadian marker rhythm - constant routine protocols have shown circadian variation in blood pressure and thyroid-stimulating hormone (TSH), whereas daily patterns in prolactin, human growth hormone (hGH), and parathyroid hormone (PTH) are sleep-wake dependent. In addition, many aspects of cognitive function, including reaction time, cognitive processing speed, math processing speed and accuracy, visual search abilities, executive function/ decision-making skills, as well as alertness, sleepiness, mood, hunger, and appetite are influenced by time awake and/or circadian time of day.

Findings from forced desynchrony protocols have shown circadian rhythms in physiology such as blood pressure, epinephrine, norepinephrine, heart rate, platelet aggregability, glucose tolerance in response to meals, EEG activity during wakefulness and sleep, susceptibility to presyncope, and periodic limb movements. Some of these outcomes, including EEG and performance, show changes that are dependent upon the levels of sleep homeostasis and circadian phase and their interaction Building on this, recent research has focused on the effects of sleep and circadian manipulations on human metabolomics and transcriptomics in an effort to elucidate altered mechanisms and biochemical pathways by which these manipulations confer increased risk for disease states and to identify potential health and disease biomarkers.

Light entrainment

Exposure to light in the early biological night induces a phase delay shift of human circadian rhythms, whereas exposure to light in the late biological night induces a phase advance shift.

Circadian responses to light can be enhanced by increasing the intensity or duration of the light stimulus, using short-wavelength light, or exposing oneself to dim light prior to the resetting stimulus.

The phase-resetting effects of light on circadian rhythms are greatest near the early part of a continuous light stimulus, as compared to the later part.

The human circadian system can be reset and entrained by exposure to electrical lighting including ordinary room light.

The circadian timing of sleep and other rhythms is modulated by exposure to electrical lighting and natural lighting under real-word conditions.

The circadian system provides an internal representation of day and night, which allows the body to anticipate daily changes in the environment. When a person is normally entrained to the solar day, the circadian system therefore facilitates the transition to and from sleep, ensuring that a consolidated period of sleep occurs at night. If the circadian clock becomes misaligned with light-dark cues, impaired cognitive function and sleep disturbances can arise. The circadian system also temporally coordinates metabolic activity and organ function, thus ensuring appropriate internal synchrony with rest-activity patterns and feeding cycles. The process of circadian entrainment is therefore critical for maintaining normal human performance, sleep behavior, and energy balance.

The retinal photoreceptors that mediate light resetting of circadian rhythms are distinct from those that mediate pattern-forming vision. In humans, this was first suggested by the observation that some blind individuals with total loss of rod and cone function exhibit melatonin suppression responses to light and can entrain normally under natural conditions. It was later discovered that the retinohypothalamic projection to the SCN originates from a small subset of retinal ganglion cells that expresses the photopigment melanopsin. The melanopsin cells are intrinsically photosensitive and respond preferentially to blue light, but can be activated indirectly by rods and cones, as shown in rodents and in macaques with trichromatic vision similar to humans. It is therefore likely that both rod-cone photoreceptors and melanopsin contribute to circadian light responses in humans.

Exposure to light in the early part of the biological night elicits a phase delay shift of the human circadian system, whereas exposure to light in the late biological night elicits a phase advance shift. Both the phase and amplitude of the circadian clock determine the type of resetting response that occurs. Type-0 resetting is characterized by large phase shifts of up to 12 h and occurs via prior reduction of circadian clock amplitude. By comparison, Type-1 resetting is characterized by small phase shifts of only a few hours with little or no reduction in amplitude of the circadian pacemaker.

Exposure to intermittent bright light represents an alternative and perhaps more efficient approach for resetting human circadian rhythms than exposure to continuous light.

Melanopsin-dependent responses exhibit peak sensitivity to ~480-nm light, which corresponds to the blue portion of the visual spectrum. By comparison, the photopic visual system in humans, which is responsible for mediating color vision, is most sensitive to 555-nm green light.

For stable entrainment to occur, the phase of the circadian clock (ϕ) must be reset by an amount that is equivalent to the difference between the endogenous period length (τ) and the imposed period of the environmental synchronizer (T).

The temporal relationship between circadian phase of a rhythm and environmental time is referred to as the phase angle of entrainment. The phase angle of entrainment correlates strongly with circadian period in humans, such that plasma melatonin levels rise later (i.e., closer to bedtime) in individuals with a longer circadian period. Therefore, individual differences in circadian period are thought to contribute to differences in chronotype.

If the circadian clock is synchronized with solar time rather than social time, there should be a small but systematic difference in the timing of sleep-wake behavior from east to west. Exposure to natural lighting therefore influences the timing of sleep-wake in humans, despite our ability to manipulate our own lighting environment using electricity.

Light exerts a powerful influence on the human circadian system, with important implications for the timing of behavioral and physiologic rhythms. Exposure to natural lighting normally synchronizes the human circadian system such that sleep occurs primarily at night.

Phase resetting refers to the adjustment of the timing of circadian pacemaker via perturbations such as bright light administration.

Scientific Background of Discoveries of Molecular Mechanisms Controlling the Circadian Rhythm – nobelprize.org

The 2017 Nobel Prize in Physiology or Medicine is awarded to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their discoveries of molecular mechanisms that control circadian rhythms. Circadian rhythms are driven by an internal biological clock that anticipates day/night cycles to optimize the physiology and behavior of organisms. Observations that organisms adapt their physiology and behavior to the time of the day in a circadian fashion have been documented for a long time, but the existence of an endogenous circadian clock would only finally become established well into the 20th century.

In 1971, Seymour Benzer and Ronald Konopka identified mutants of the fruit fly Drosophila that displayed alterations in the normal 24h cycle of pupal eclosion and locomotor activity. Experiments suggested that the mutations involved the same gene, later named period. A decade later, Hall and Rosbash, collaborating at Brandeis University, and Young, at Rockefeller University, isolated and molecularly characterized the period gene. However, its structure and sequence did not immediately suggest a molecular mechanism for the circadian clock. A series of breakthroughs, including the identification of other genes that partner with period, from Hall, Rosbash and Young eventually led to the notion of a Transcription-Translation Feedback Loop (TTFL). In this mechanism, the transcription of period and its partner gene timeless are repressed by their own gene products – the PERIOD (PER) and TIMELESS (TIM) proteins, generating an autonomous oscillation.

At the time, a transcriptional mechanism was not obvious, and the discovery of the selfsustained circadian TTFL was a new paradigm. Further studies revealed a series of interlocked transcription-translation feedback loops, together with a complex network of reactions. These involve regulated protein phosphorylation and degradation of TTFL components, protein complex assembly, nuclear translocation and other post-translational modifications, generating oscillations with a period of ~24 hours. Circadian oscillators within individual cells respond differently to entraining signals and control various physiological outputs, such as sleep patterns, body temperature, hormone release, blood pressure, and metabolism. The seminal discoveries by Hall, Rosbash and Young have revealed a crucial physiological mechanism explaining circadian adaptation, with important implications for human health and disease.

What makes us tick?

A key feature of life on Earth is its capacity to adapt to the environment. Different geographical locations have different environments and organisms adapt to the conditions that are prevalent at their location to enhance their survival. However, at any given location, profound changes in environmental light and temperature occur daily as a consequence of the rotation of the Earth on its axis. To adapt to such changes, most organisms have evolved an internal biological clock that anticipates day/night cycles and helps them optimize their physiology and behavior. This internally generated daily rhythm is known as “circadian”, from the Latin words circa meaning “around” and dies meaning “day”.

Circadian rhythms are ancient and conserved throughout evolution. They are known to exist in life forms from unicellular cyanobacteria and protozoans to all multicellular organisms, including fungi, plants, insects, rodents and humans. The building blocks of a circadian system consist of a self-sustained 24-hour rhythm generator or oscillator, setting or entraining mechanisms that link the internal oscillator to external stimuli (referred to as zeitgebers, i.e. timekeepers), such as light, and output mechanisms to allow the timely scheduling of physiological processes.

From rhythms to clocks

Observations that organisms adapt their physiology and behavior to the time of the day in a circadian fashion have been documented for a long time and are commonly agreed to have begun with the observation of leaf and flower movements in plants. For example, the leaves of mimosa plants close at night and open during the day. In 1729, the French astronomer Jean Jacques d’Ortous de Mairan placed a mimosa plant in the dark and observed that the leaves still opened and closed rhythmically at the appropriate time of the day, suggesting an endogenous origin of the daily rhythm.

About two hundred years later, the German plant physiologist and pioneer of circadian rhythm research, Erwin Bünning, painstakingly connected the leaves of a bean plant to a kymograph and recorded the movements of the leaves during normal day/night cycles and under constant light conditions. He observed that the rhythm of leaf movement persisted.

The question of whether circadian behaviors in plants and animals were governed by an endogenous clock, or were a mere reaction to external stimuli of a circadian nature, would be hotly debated for decades. Eventually, the existence of an endogenous circadian clock would finally become established well into the 20th century.

Heritability of circadian rhythms and clock genes

With time, many relevant physiological properties besides periodic leaf movements were found to be controlled by the physiological clock and the inheritance of circadian rhythms began to be considered as the product of natural selection. Erwin Bünning's classical studies in the 1930s showed that circadian rhythms in plants can be inherited despite parent plants being exposed to non-circadian light periods and that crosses between strains with varying periods yielded plants with intermediate periods. By the mid1960s, a community of chronobiology researchers investigating biological clocks was well established and the concept of clock genes began to be contemplated.

It was at about this time that Seymour Benzer and his student Ronald Konopka, working at the California Institute of Technology, embarked on studies to identify mutant fruit flies with altered circadian phenotypes.

Using a classical chemical-based mutagenesis strategy, Benzer and Konopka isolated three different strains of mutant flies showing alterations in the normal 24h cycle of pupal eclosion and locomotor activity (Konopka and Benzer, 1971). One mutant was arrhythmic, another had a shorter period of 19h, and a third had a longer period of 28h. Mapping experiments, using the genetic markers known at the time, roughly localized all three mutants to the same region of the X chromosome of the fruit fly. Importantly, complementation tests suggested that the three mutations involved the same gene, later named period.

However, the period gene would not be molecularly cloned and sequenced until the mid1980s through the work of Jeffrey Hall and Michael Rosbash, collaborating at Brandeis University, and Michael Young, at Rockefeller University. The first clock gene was thereby isolated, and its structure was molecularly characterized. However, neither the original genetic identification of period nor the cloning and sequencing of its cDNA pointed to a molecular mechanism for the circadian clock.

The Transcription-Translation Feedback Loop

In the years following the cloning of period, several models were proposed to explain how its protein product PER might function to produce circadian oscillations. A "membrane gradient" model was proposed in which PER was envisioned to function like a pump to build a gradient across the membrane. In another model, the PER protein was proposed to be a proteoglycan that brings cells together, thereby facilitating the formation of inter-cellular connections through gap junctions.

Current working models of the circadian molecular clockwork are highly complex and include many additional components which, collectively, contribute to its robustness and circadian

periodicity (Hardin, 2011). Importantly, as transcription and translation reactions are typically rapid, substantial delays must be imposed on the core TTFL mechanism to generate 24h oscillations. This is achieved by a complex network of reactions involving regulated protein phosphorylation and degradation of TTFL components, protein complex assembly, nuclear translocation and other post-translational modification.

Circadian clocks in other organisms

TTFL mechanisms are also an underlying principle of circadian clocks in other multicellular organisms. However, in cyanobacteria, a different type of transcription-independent circadian oscillator has been described that depends on sequential protein phosphorylation events. Nevertheless, these results suggest that additional mechanisms for generating circadian oscillations may also exist in mammalian cells.

Entrainment and synchronization of biological clocks

The circadian program is regulated at both a central and peripheral level. In mammals, the central pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and functions as the master circadian clock. The retina receives photic input and relays this information to the SCN, which synchronizes its own neuronal cellular clocks. The central clock regulates circadian rhythms across the entire body via humoral factors and the peripheral autonomic nervous system. However, the capacity for circadian gene expression is widespread throughout the body and most peripheral organs and tissues can express circadian oscillations in isolation.

Peripheral clocks can be synchronized both by the SCN and by environmental cues, including feeding, physical activity and temperature. Peripheral clocks in different tissues control relevant physiological outputs, such as glucose production, fat storage and release of hormones. The relationship between the central and peripheral clocks, and the multiple ways by which local and external cues affect them, is an active area of research open to new discoveries.

Circadian biology and human health

Chronobiology has an impact on many aspects of our physiology. For example, circadian clocks help to regulate sleep patterns, feeding behavior, hormone release, blood pressure and body temperature. Molecular clocks also play critical roles locally in many tissues. Ablation of clock genes in animal models results in arrhythmic production of hormones, such as corticosterone and insulin. Clock genes also exert a profound influence on metabolism through the control of gluconeogenesis, insulin sensitivity and systemic oscillation of blood glucose. Sleep is vital for normal brain function and circadian dysfunction has been linked to sleep disorders, as well as depression, bipolar disorder, cognitive function, memory formation and some neurological diseases. Efforts are underway to develop approaches in chronobiology and pharmacology to modify the period, phase or amplitude of circadian clocks to improve human health.

Conclusions

The discovery of self-sustained transcription/ translation feedback loops as the central component of the molecular mechanism by which clock genes control circadian oscillations in cells and tissues has led to a new paradigm in our understanding of how organisms anticipate and adapt to the regular daily environmental cues such as light. Since the seminal discoveries by the three laureates, elucidating a fundamental physiological mechanism, circadian biology has developed into a vast and highly dynamic research field, with important implications for our health and wellbeing.

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