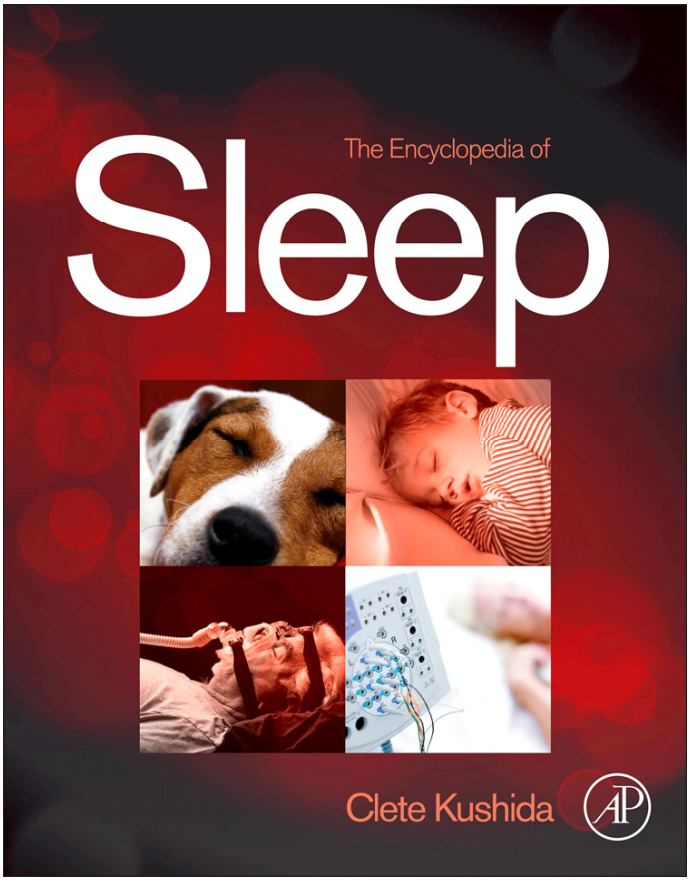

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The Influence of Light, Exercise, and Behavior upon Circadian Rhythms*

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Glossary

Circadian: Occurring regularly at about 24-h intervals, even in the absence of environmental periodicity.
Entrainment: Coupling of an endogenous rhythm to an environmental oscillator with the result that both oscillations have the same frequency.
Free-running: Continuing with an endogenous periodicity consistently different from any known environmental schedule.
Pacemaker: A functional entity capable of self-sustained oscillations which synchronize other rhythms.
Period: Duration of one complete cycle in a rhythmic variation.
Phase: Instantaneous state of an oscillation relative to a reference point within the oscillation.
Phase-response curve (PRC): The 24-h profile of an organism's phase shifts in response to environmental signals.
Phase shift: Displacement of an oscillation along the time axis. (By convention, advances are represented as positive numbers, and delays as negative ones.)
Suprachiasmatic nucleus (SCN): Anatomical structure serving as the master circadian clock in mammals. It is a group of hypothalamic neurons situated above the optic chiasm that receives external phase information via the retina, and possesses intrinsic cell-autonomous circadian timing capability.
Synchronization: State of a system when two or more variables exhibit periodicity with the same frequency and specifiable acrophase and phase relation.
Zeitgeber: Timing cue influencing the phase of a biological rhythm.

Introduction

A circadian rhythm is an endogenously driven pattern of behavior or physiology in an organism that repeats periodically approximately each day. Such patterns exist in organisms throughout evolution, from bacteria to plants and animals. In metazoans, the prototypical example of a behavior that demonstrates a circadian rhythm is the sleep–wake cycle, with its alternating intervals of activity and with restfulness that recur with a periodicity approximating the 24-h day–night cycle. Not only do circadian rhythms govern sleeping patterns, but in mammals they also play a part in controlling body temperature, hormone secretion, blood pressure, metabolism, and many other functions.

Concept of Biological Clocks

The mechanism underlying circadian rhythms is a biological 'circadian clock' Conceptually, circadian clocks can be divided into three quasi-independent processes: input pathways, which relay environmental information to a central pacemaker (clock); a circadian pacemaker, which generates the oscillation; and output pathways, through which the pacemaker regulates molecular and biochemical pathways that lead to rhythms in physiology and behavior. Circadian clocks, as opposed to other biological oscillators such as the cell cycle or the menstrual cycle, meet four general criteria: (i) Their period length, that is, the time taken for one complete cycle, is about 24 h (hence the word 'circadian,' from the Latin *circa diem*, or 'about a day'). (ii) The rhythm must persist under constant environmental conditions, maintaining its periodicity of approximately 24 h. (iii) The rhythm exhibits temperature compensation, keeping roughly the same period length at different environmental temperatures. A normal biochemical reaction will approximately double its speed with every 10 °C increase in temperature, but circadian clocks do not. (iv) The rhythm can be synchronized by environmental cues, commonly called Zeitgebers (German for 'timing cues'). This synchronization, or entrainment, allows the clock to change its phase, or internal time, to better match local external time.

Thus, environmental Zeitgebers induce molecular changes in the circadian oscillator, which adjust it to the environment. One of the most potent of these cues is light, which is capable of changing clock phase in either direction depending upon the time it is perceived, and thereby altering the timing of daily behaviors such as rest–activity cycles and feeding. Interestingly, however, both behavior (i.e., exercise) and feeding themselves can shift clock properties at a molecular level. The result is an oscillator that responds both to the exigencies of the environment and the reality of an organism's behavior in it. In the following pages, we first outline the mammalian circadian oscillator at a molecular and systemic level, and then summarize current knowledge about its entrainment.

Molecular Basis of Circadian Clocks

Knowledge of the molecular components of the circadian clock was first established in the fruit fly *Drosophila melanogaster*, where mutation of the gene *period* (*per*) caused altered circadian rhythms. Subsequent isolation of the affected gene showed that

*Note: The format of this article to align with the publishing guidelines explicitly excluded referencing, and instead specified a further reading list. Clearly, the contributions cited in this article were the product of hundreds of individuals in dozens of laboratories, and we regret being unable to cite their contributions individually.

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both *per* RNA and protein are expressed rhythmically. Evidence that the PER protein product repressed transcription of its own gene led to the theory that regulation of the circadian rhythm on a molecular level is controlled by interlocking autoregulatory feedback loops of transcription and translation that involve a set of clock genes and their protein products.

In mammals, the molecular setup of the circadian clock may be schematically imagined as consisting of positive and negative elements. Positive components include the two transcription factors CLOCK (circadian locomotor output cycle kaput) and BMAL1 (Brain and Muscle ARNT like 1) and their homologs. These transcription factors form heterodimers through their PAS and HLH regions and bind *cis*-acting E-Box sequences (consensus sequence: CACGTG) present in the promoter regions of many clock and clock-controlled genes. Among the genes so activated are the three period genes (*mPer1–mPer3* in the mouse) and two cryptochrome genes (*mCry1* and *mCry2*), which are the key negative components of this feedback loop.

To close this feedback loop, *Per* and *Cry* transcripts are translated in the cytoplasm several hours later and form multimeric complexes of unknown stoichiometry that are translocated back into the nucleus. There, they negatively regulate their own expression by repressing the transcriptional activity of CLOCK/BMAL1. A subsequent decline in PER and CRY protein levels through both lack of transcription and active posttranslational modification, proteasome targeting, and degradation finally leads to a reactivation of CLOCK/BMAL1-driven transcription and initiation of the next cycle. This basic feedback loop recurs autonomously on a daily basis.

Other clock genes play a role in stabilizing the circadian rhythm by forming additional interlocked feedback loops. For example, CLOCK/BMAL1 heterodimers regulate not only transcription of *Per* and *Cry* loci, but also of retinoic acid-related orphan nuclear receptor genes *Rev-Erbα* and *RORα*. The REV-ERBα protein represses *Bmal1* transcription by binding to retinoic acid-related orphan receptor-response element (RORE) sequences in the *Bmal1* promoter. Similarly, RORα also binds the RORE sequences in the *Bmal1* promoter, but in contrast to REV-ERBα, RORα activates *Bmal1* transcription. Therefore transcription of *Bmal1* is the result of the competition between REV-ERBs and RORs at their specific response elements (RORE), interconnecting positive and negative feedback loops of the clock.

In addition to transcriptional regulation, posttranslational mechanisms such as protein phosphorylation have been found to be critical in generating circadian oscillations. For instance, casein kinase ε and δ (CK1ε/δ) are essential components of the feedback loops that generate circadian rhythm in mammals. CK1ε phosphorylates PER, CRY, and BMAL1 proteins. CK1δ, a close paralog of CK1ε, has also been found to be associated with PER/CRY complexes and may therefore perform a similar function. Although phosphorylation of clock proteins at different sites leads to varying effects, one key role of this modification is to favor ubiquitinylation and subsequent proteasome-mediated degradation of proteins thus modified.

Master and Peripheral Clocks

From single-celled organisms to mammals, the basic circadian clockwork is cell-autonomous and present in most cells and tissues. Thus, individual cells (neurons, fibroblasts, hepatocytes, etc.) show circadian oscillations of gene expression, even in isolation. Nevertheless, in complex organisms such as mammals, these clocks are arranged in a hierarchy. The master pacemaker of the mammalian circadian clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The SCN is a heterogeneous tissue comprised of at least two broad regions. SCN neurons of the ventrolateral part (core) are heavily innervated by the retinohypothalamic tract (RHT), and possess the unique property of inducing expression of several genes, including *Per1* and *Per2*, in response to light stimulation. However, their intrinsic circadian oscillations are weak. In contrast, cells of the dorsomedial part (shell) receive most of their information from the hypothalamus and limbic areas as well as from the core, and show strong autonomous rhythmicity but only weak activation by photic input.

SCN-lesioned animals lose global circadian rhythmicity of diurnal behavior and physiology in constant conditions, for example, corticosterone rhythm, drinking behavior, and locomotor activity, and SCN grafts restore these rhythms. Thus, the SCN is responsible for entraining and synchronizing cellular clocks throughout the body. This mechanism will be discussed in more detail below. First, though, comes the question of what entrains the SCN, and how.

Phase Shifts and Phase Response Curves

Light is considered the strongest Zeitgeber for the SCN. Every day, it resets the phase of the endogenous circadian clock and the linked oscillations in physiology and behavior to exactly match the 24-h day (up to a maximum of approximately 2 h per day in humans), even if the endogenous 'free-running' period of the internal clock is shorter or longer. Importantly for this result, the effect of light on the SCN varies depending on the time at which it is perceived. Thus, resetting of the circadian system by light exposure at most times during the subjective day has little or no effect on the resetting of the clock. By contrast, light exposure in the late night and early morning leads to phase advances (so that locomotor activity will start earlier the next day) and light exposure in the evening and early night delays the clock (so that activity will start later the next day).This phase dependence of light-induced phase shifts can best be visualized as a phase response curve (PRC), which plots phase shifts of a circadian rhythm as a function of the circadian phase that a stimulus is given. The human PRC to bright light pulses is shown in **Figure 1(a)**. Although biological mechanism may vary considerably, all known environmental factors that phase-shift the clock (both natural and artificial) do so in a time-dependent fashion, though the shape of the PRC varies. For example, the PRC of the hormone and drug melatonin is shown in **Figure 1(b)**.

Natural environmental inputs to the circadian oscillator are generally divided into photic (light) and nonphotic (exercise, social interaction) categories. As we shall see, these stimuli reach the SCN and peripheral clocks via very different pathways, but in each case the result is rhythmic physiology and behavior that is optimally entrained to the environment.

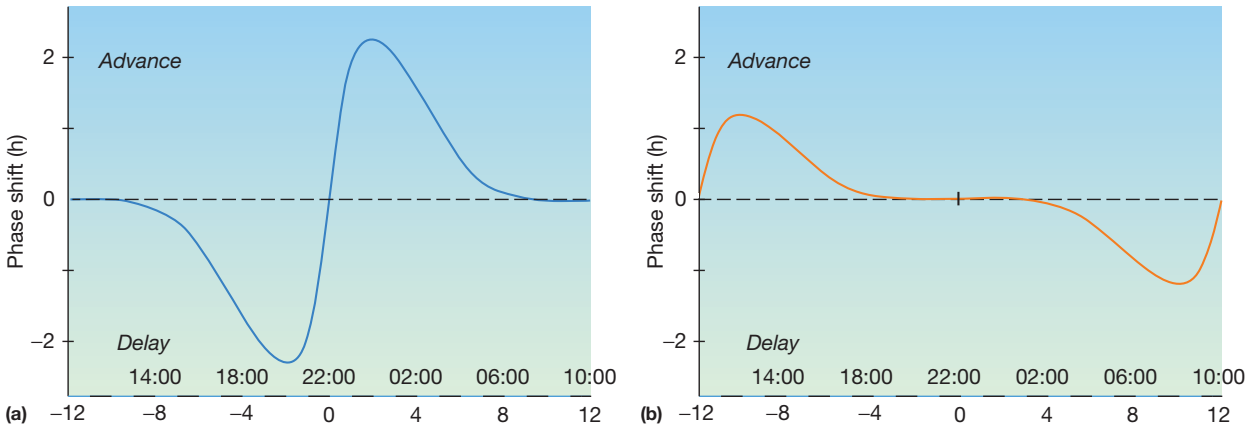

Figure 1 Schematic representation of the phase response curves (PRCs) to light and melatonin. (a) Light exposure in the early evening leads to a delay of circadian rhythms, whereas light exposure in the early morning causes phase advances. On the *X*-axis, the time point 0 corresponds roughly to the minimum of the body temperature. For convenience, conventional time of day is also plotted. During daytime, there exists a 'dead zone' during which light exposure has little effect on circadian phase shifting. (b) The PRC to melatonin is about 12 h out of phase with that of light and exhibits less strong phase shifting, represented by the smaller amplitude. Adapted from Lewy AJ, Bauer VK, Ahmed S, (1998) The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. *Chronobiology International* 15: 71–83; Khalsa SB, Jewett ME, et al. (2003). A phase response curve to single bright light pulses in human subjects. *The Journal of Physiology* 549(Pt 3): 945–952.

Light's Path to the Mammalian Circadian Clock

Light is the strongest timing signal, but its pathway to the SCN is unconventional. In the last century, it was thought that it went through the photoreceptive rods and cods found within the eye. However, studies later demonstrated that mice lacking all rods and cones were visually blind, but the circadian system remained responsive to photic signals.

In lower metazoans such as *Drosophila*, the single cryptochrome protein (dCRY) acts as a blue light photoreceptor. In contrast to *Drosophila*, the CRY proteins in mammals play a primary role as essential transcriptional repressors of the circadian clock, but a possible auxiliary role as a photoreceptor remains highly controversial. Tellingly, *mCry1*/*mCry2* double-knockout mice are still able to induce PER expression upon a light pulse. In addition, mammalian *Cry1* and *Cry2* have been shown to negatively regulate PER1 transcription independent of light. However, mice depleted of rods and cones and lacking both cryptochromes were no longer able to react to light, at least as measured by acute *cFos* transcription (a marker of neural activity). Confirming CRY as a mammalian circadian photoreceptor is difficult because animals without CRY show arrhythmic behavior. However, since animals with functional cryptochromes but lacking rod and cone cells and the retinal ganglion pigment melanopsin are functionally blind for the circadian oscillator and unable to be light-entrained, any physiological requirement for cryptochromes likely lies outside light perception per se.

In humans, because of the potential health threat posed by shiftwork, light entrainment has been intensely studied in spite of the arduousness of performing such experiments. At high irradiances of light, the human PRC shows no 'dead zone,' and can respond to light at all times of day. Blue and green wavelengths appear most potent. At lower intensities, the effects of light show both wavelength and time dependence. As in other species, blindness equivalent to enucleation results in complete failure to entrain to light signals, consistent with an ocular process.

Mammals receive external light information to the circadian clock mainly through traditional rods and cones as well as specialized retinal ganglion cells (RGCs) that express the photopigment melanopsin. Exposure to light leads to depolarization of RGCs, which innervate the SCN via the RHT. The monosynaptic RHT fibers end directly on SCN neurons in the ventrolateral part of the nucleus, releasing the excitatory neurotransmitter glutamate and the neuropeptide pituitary adenylate cyclase-activating protein (PACAP). Importantly, however, glutamate is released at both early and late night, whereas PACAP secretion seems to be important mainly during late night.

Molecular Mechanisms of Light-Induced Phase Shifts

The RHT efferents, upon stimulating SCN neurons, induce cellular signal transduction cascades that are ultimately able to shift the SCN clock in both directions. Two different theories have emerged about how this happens, and in fact current evidence suggests that both are correct. On one hand, light might affect directly and uniformly the abundance of a clock protein, for example, by increasing it. Depending upon whether the cell is trying to synthesize this clock protein (at one time of the circadian cycle) or to degrade it (at the opposite time), relative clock phase will advance or delay. On the other hand, light might induce alternative signaling cascades at different times of day which affect the clock in different ways.

In either case, response to photic stimulation of ganglion cells in the RHT is similar: glutamate and PCAP are released from their terminals and activate SCN cells. *In vivo*, application of both glutamate and PACAP into the SCN shifted the circadian clock with a PRC similar to that of light. Additionally, the effects were mimicked by injecting glutamate into the SCN or

applying it *in vitro* through 2-Amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors. Application of glutamate receptor antagonists blocked light-induced phase shifts respectively. Finally, injections of NMDA into the SCN of animals *in vivo* during their subjective night time induced photic-like behavioral phase shifts. Thus, for phase shifts in either direction, the initial stimulus is likely glutamatergic.

The result of this signaling is Ca^{2+} influx through NMDA activation. The downstream actions of calcium, discussed further below, likely include activation of calcium/calmodulin, MAP kinase, and other kinases, which results in the end in the phosphorylation of Ca^{2+}/cAMP-response element binding protein (CREB).

One important result of the activation of Ca^{2+} and calcium/calmodulin-dependent kinase (CaMKII) is phosphorylation of nitric oxide synthase (NOS), which in turn produces nitric oxide (NO). The subsequent NO production provokes phase shifts at early and late night both *in vivo* and *in vitro*. However, the mechanisms of NO seem to depend on the time of the light pulse. Downstream of the CamKII/NOS pathway, divergent signaling pathways might explain differences between phase delays and advances. For example, Ding and coworkers found that light-induced phase delays involve intracellular calcium release through ryanodine receptors in the circadian clock, which is restricted to the early night. By contrast, in the late night, NO released by NOS activates the soluble form of guanylyl cyclase (GC), leading to an increase in cGMP levels and the eventual activation of cGMP-related protein kinase (PKG) to produce phase advances. Consistent with this data, inhibition of the cGMP-related protein kinase (PKG) suppresses the phase-advancing effects of light and glutamate *in vivo* and *in vitro*. Similarly, pharmacological inhibition of phosphodiesterase 5 (PDE5), an enzyme that catalyzes cGMP degradation, increases cGMP levels and thereby enhance light-induced phase advances of circadian rhythms. Thus, the selective PDE5 inhibitor sildenafil (better known as Viagra) not only increased light-induced phase advances but also accelerated reentrainment to a 6-h phase advance in the light–dark cycle in an animal model of jetlag, but had no effect on phase delays.

Another substrate for phosphorylation by CamKII is CREB. Phosphorylated CREB (pCREB) protein can then bind to its response element, the CRE, within the promoter region of target genes, such as *c-fos* and *mPer1*/*mPer2*, to activate their expression. A similar CREB pathway leads from the other RGC neurotransmitter PACAP. Light exposure in the late night leads to activation of GS-coupled PAC1 receptors via PACAP, which signals to the MAPK (mitogen-activated protein kinase) pathway mediated by a signaling cascade including cAMP production by adenylylcyclases (AC). The upregulation of MAPK (ERK1 and 2) phosphorylation leads to the activation of CREB.

One effect of light-induced activation of CREB is increased expression of *mPer1* (and sometimes *mPer2*), whose promoter regions contain CREB elements. However, thousands of genes contain such elements, and hundreds are activated in any given tissue. Thus, though much has been learned, additional work will be required to further elucidate the signaling cascades that underlie phase shifts during both early and late night.

Other Timing Cues: Hints from Neuromorphology

Although light is the principal timing cue (Zeitgeber) of the mammalian circadian oscillator, the SCN receives input from other brain regions, hinting that other timing cues also exist. The three major input pathways of the SCN are the RHT, responsible for transmitting light information as described above; the intergeniculate leaflet (IGL) via the geniculohypothalamic tract (GHT); and serotonergic input from the raphe nucleus.

Next to the RHT, the second most important afferent pathway to the SCN comes from the GHT originating from the IGL. In addition to receiving input from other brain regions, the GHT is also innervated by RGCs, presumably transmitting the same information as through the RHT. The main important transmitters within the IGL are the neuropeptide Y (NPY) and γ-aminobutyric acid (GABA). Several studies have shown that the IGL mediates photic responses as well as nonphotic entrainment of circadian rhythms and receives additional input from the raphe nuclei.

The third set of neuronal projections are mediated indirectly by the raphe nuclei, containing serotonin (5-HT), which mainly innervates the SCN core. Some efferent projections reach the shell, and could mediate nonphotic interactions.

Nonphotic Entrainment: Exercise

Although light is considered the most potent behavioral *zeitgeber* in mammalian clocks, many studies report evidence that nonphotic stimuli such as exercise and behavior are also able to synchronize and entrain mammalian circadian rhythms. Interestingly, as we explain below, these signals act very potently upon peripheral circadian oscillators, even as the central clock remains light-driven under normal circumstances.

Scheduled physical activity such as wheel running and forced treadmill running in nocturnal rodents has been reported to influence the circadian timing system. Under constant conditions, periodical induction of physical activity by wheel running or forced treadmill running shifted or entrained the free-running period. Additionally, voluntary exercise shortened the free-running period in mice and rats. Novelty-induced running for a 3-h pulse at different circadian times revealed a PRC in the Syrian hamster, with phase advances induced in the subjective day (the hamsters' rest period) and a small delay in the late subjective night. These observations confirm that nonphotic stimuli such as exercise are able to entrain the circadian clock in nocturnal rodents, although some species (e.g., hamsters) are much more responsive than others (e.g., rats).

Several studies using single bouts of exercise in humans demonstrated that the timing of exercise is associated with phase shifts of the biological clock. In these studies, circadian phase was determined by measuring the phase of the circadian hormone melatonin, once in the absence of physical exercise and once with physical exercise of 1–3 h duration at various intensities. The PRC derived from these studies shows circadian phase advances by physical exercise in the evening and delays in the subjective night, consistent with studies in rodents. Interestingly, however, phase determination was made at one and two circadian cycles immediately after the physical

exercise. A phase advance was seen one cycle after the exercise session but not on the second cycle. Therefore the changes in phase might be due to other variables, such as increased metabolism rather than the exercise itself. (As discussed below, metabolic effects are also potent Zeitgebers, but especially so for peripheral oscillators. Were non-SCN oscillators more potently affected than the SCN itself, transient phase-shifting would be one possible outcome.)

So far, no mechanism has been proven for exercise-dependent phase-shifting. One plausible candidate, however, is body temperature. Increased locomotor activity such as exercise is associated with a rise of body temperature in mammals. Under normal circumstances, the core body temperature varies 1–4° over the time course of 24 h in mammals, showing the largest dip during the resting period. The effect of locomotor activity on the body temperature is higher during the resting period (minimum of motor activity) than during the active period in mice. On a cellular level, temperature cycles have been shown to entrain peripheral cells and organs, even as the SCN remains in phase with light. It has been postulated that this relates to an inherent inability of the SCN to respond to temperature, or alternatively because the SCN has different intercellular coupling properties than other tissues. In peripheral tissues, however, it is clear that very slight temperature changes are potent Zeitgebers, likely mediated at least in part by circadian activation of heat shock factor 1 (HSF1), the central transcription factor of the heat shock response.

Nevertheless, body temperature is unlikely to be the sole mechanism of exercise-mediated entrainment. Clocks in HSF1-deficient cells and mice still respond, albeit in attenuated fashion, to temperature signals. Moreover, injections of some drugs that cause large increases in locomotor activity and corresponding increases in body temperature (e.g., Triazolam, a short-acting benzodiazepine) induce large phase advances in the circadian clock, but do so in both free-running animals (which exercise in response to drug administration) and restrained control animals (which do not). Thus, several authors have postulated that 'social cues' independent of actual exercise can also act as circadian Zeitgebers. In this regard, physical exercise affects many physiological parameters, which are highly interconnected with each other and might feedback to the SCN in order to regulate circadian rhythm. For example, some nonphotic stimuli can shift circadian rhythms through the geniculo-hypothalmic tract by serotonin (5-HT) and/or neuropeptide Y (NPY) inputs to the SCN circadian clock. Given the widely disparate mechanisms proposed for nonphotic phase-shifting, further studies are clearly needed to better understand clock resetting by physical exercise and the relation between photic and nonphotic entrainments.

Feeding Behavior as a Zeitgeber

Normally, the SCN regulates resting and activity periods in mammals, enabling locomotor activity and eating during the waking phase. Nevertheless, if food is restricted to a particular time of day, laboratory rodents will soon anticipate this timing and begin locomotor activity prior to food availability. Interestingly, this behavior is also present in SCN-lesioned animals. Therefore, it is hypothesized that in addition to the light-entrainable SCN, another clock exists which can be entrained by food, namely, the food entrainable oscillator (FEO). Anatomically, the FEO might include the dorsal medial nucleus (DMH) of the hypothalamus, the paraventricular nucleus of the hypothalamus, and the cerebellum. Opinions about this organization are divided, however. For example, studies of *mPer1* and *mPer2* expression in the dorsomedial hypothalamic nucleus of rats and mice suggest that the phase of food-anticipatory activity is not directly regulated by this brain area but rather modulated by the motivational state of the animal. Moreover, the molecular basis of the FEO also remains unclear, because some 'traditional' clock genes seem necessary for FEO-driven anticipatory activity and others do not.

Perhaps separate from its ability to entrain behavior, timing of food intake has been shown to play an important role along with temperature in the entrainment of peripheral clocks. Normally, feeding occurs coincident with daily activity. If, however, food availability is restricted to the quiescent period, circadian clocks in peripheral tissues will become uncoupled from the central pacemaker in the SCN and will change to match food timing, though the speed and the degree to which an organ changes its circadian phase varies among organs. Unlike the FEO discussed above, which regulates food-anticipatory activity, food-dependent peripheral oscillator reentrainment is likely a cell- or organ-autonomous process resulting in global changes in the phase of clock gene expression.

The mechanism of this entrainment remains incomplete. Hormonal effects, at least through glucocorticoids, are not involved; in fact, organs engineered to lack glucocorticoid receptors or adrenalectomized mice surprisingly entrain more rapidly, suggesting that brain-driven rhythmic glucocorticoid secretion counteracts feeding entrainment. Metabolites associated with feeding such as insulin and glucose have been shown to be able to shift clock gene expression in cultured fibroblast cells, but the relevance of this observation *in vivo* is unknown. What is clear, however, is that a subset of clock genes in peripheral tissues – including the crucial circadian repressor *Per2* – can be systemically driven by feeding entrainment. In parallel, several clock proteins, as well as histones at clock gene loci, appear to be deacetylated by the enzyme SIRT1 in a fashion dependent upon the nicotinamide adenine dinucleotide cofactor NAD^+. Moreover, the DNA-binding activity of CLOCK/BMAL1 or NPAS2/BMAL1 clock protein heterodimers *in vitro* is also strongly affected by the ratio of NAD cofactors. Finally, the enzyme poly(ADP-ribose) polymerase 1 (PARP-1) is regulated by feeding and covalently modifies the CLOCK protein in NAD^+-dependent fashion to regulate its activity. Since metabolic state at a cellular level involves changes in the redox equilibrium of NADH and NADPH and their counterparts NAD^+ and $NADP^+$, clock protein regulation by NAD redox mechanisms could provide a posttranslational mechanism for feeding entrainment (Figure 2). Consistent with this idea, the entrainment of liver clocks to inverted feeding is delayed in PARP-1 knockout mice.

Outlook: Entrainment of and by the Clock

In this chapter, we have summarized existing data about the molecular mechanisms of entrainment of the circadian clock

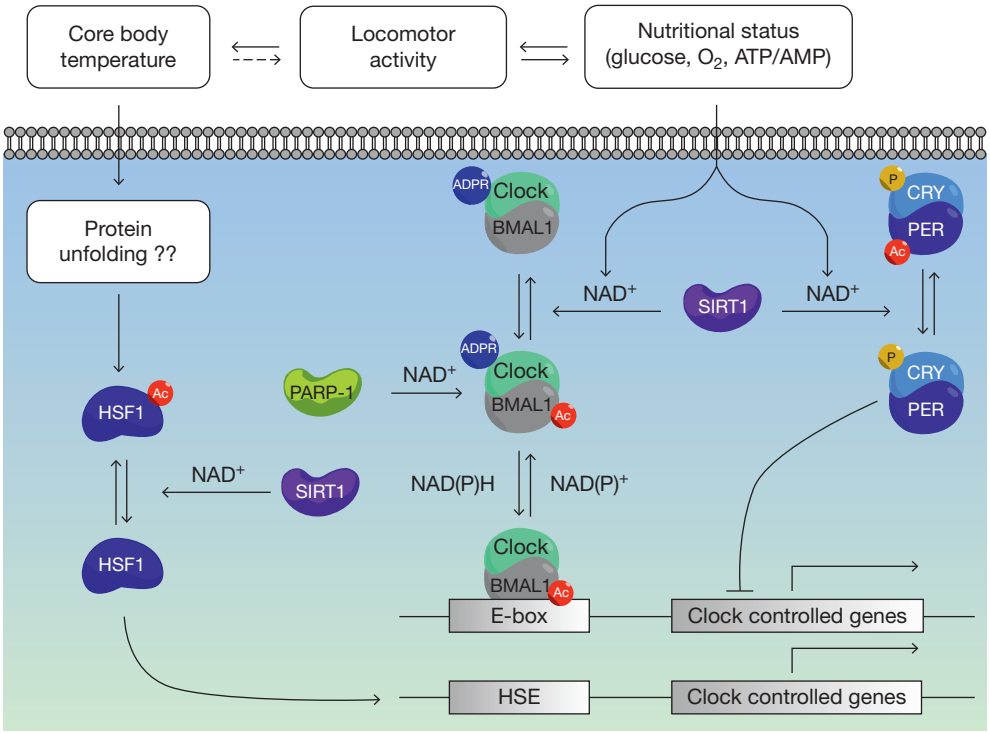

Figure 2 Molecular regulation of core clock genes by food and activity. Regulation by nutrition: The cellular redox potential of NAD^+/NADH and $NADP^+$/NADPH is regulated by the metabolic state of the organism, which in turn is dependent upon food uptake and physical activity. The redox sensor SIRT1 deacetylates core clock genes such as BMAL1 (which can be acetylated by CLOCK) and PER2 in a NAD^+-dependent fashion. This deacetylation leads to increased PER/CRY instability, enhancing BMAL1-mediated transcription. In addition, activity of poly(ADP-ribose) polymerase 1 (PARP-1) is regulated in a circadian- and nutrition-dependent fashion by NAD^+, and modulates CLOCK-mediated transcription by ADP-ribosylation of CLOCK. Regulation by activity: Increased locomotor activity not only affects cellular redox potential but also elevates core body temperature, resulting in HSF1 activation, perhaps via protein unfolding. HSF1 can bind to heat shock elements (HSE) in promoter regions of some clock-controlled genes. In addition to its direct effects upon clock proteins, SIRT1 also regulates activity of HSF1. Adapted from Asher G and Schibler U (2011) Crosstalk between components of circadian and metabolic cycles in mammals. *Cell metabolism* 13(2): 125–137.

to environmental light stimuli, as well as to indirect signals such as exercise and food. Through pathways mostly separate from that of light, these indirect signals probably serve normally to reinforce and even communicate SCN-driven timing to autonomous circadian clocks in peripheral organs. In the case of repeated conflict, they can rephase rhythms in these organs, and ultimately in the SCN in the absence of dominating light signals.

Interestingly, the known pathways regulating clock entrainment through indirect cues – namely, temperature and cellular redox state, though others probably remain to be discovered – are themselves normally regulated in circadian fashion by the clock. Thus, a complex feedback loop is formed through which a competition between intrinsic and environmental influences ultimately determines clock phase.

Because it exists within the highly conserved circadian machinery, it is likely that such a competition was evolutionarily advantageous. Nevertheless, recent studies have shown that chronic clock misphasing by light, exercise, and social cues (e.g., via shiftwork) is harmful in both humans and in animals. However, both the causes and the societal implications of these disturbances remain to be addressed, and will likely be the subject of much future research.

See also: **Chronobiology of Sleep:** Chronobiology of Sleep – Circadian Rhythms, Behavior, and Performance; Circadian Rhythms and Physiological Processes; Effects of Melatonin and Melatonin Agonists on Circadian Rhythms; Molecular and Genetic Bases for the Circadian System.

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