Coupling the Circadian Clock to Homeostasis: The Role of Period in Timing Physiology

Pureum Kim,[1] Henrik Oster,[2] Hendrik Lehnert,[3,4] Sebastian M. Schmid,[3,4] Nicole Salamat,[1] Johanna L. Barclay,[5] Erik Maronde,[6] Warrick Inder,[7,8] and Oliver Rawashdeh[1]

[1]School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072, Australia; [2]Institute of Neurobiology, University of Lübeck, 23562 Lübeck, Germany; [3]Department of Internal Medicine 1, University of Lübeck, 23562 Lübeck, Germany; [4]German Center for Diabetes Research, 85764 Neuherberg, Germany; [5]Mater Research Institute, University of Queensland, Brisbane, Queensland 4072, Australia; [6]Department of Anatomy, Goethe University Frankfurt, 60590 Frankfurt, Germany; [7]Faculty of Medicine, University of Queensland, Brisbane, Queensland 4072, Australia; and [8]Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia

ORCiD numbers: 0000-0002-7147-4778 (O. Rawashdeh).

ABSTRACT A plethora of physiological processes show stable and synchronized daily oscillations that are either driven or modulated by biological clocks. A circadian pacemaker located in the suprachiasmatic nucleus of the ventral hypothalamus coordinates 24-hour oscillations of central and peripheral physiology with the environment. The circadian clockwork involved in driving rhythmic physiology is composed of various clock genes that are interlocked via a complex feedback loop to generate precise yet plastic oscillations of ~24 hours. This review focuses on the specific role of the core clockwork gene *Period1* and its paralogs on intra-oscillator and extra-oscillator functions, including, but not limited to, hippocampus-dependent processes, cardiovascular function, appetite control, as well as glucose and lipid homeostasis. Alterations in *Period* gene function have been implicated in a wide range of physical and mental disorders. At the same time, a variety of conditions including metabolic disorders also impact clock gene expression, resulting in circadian disruptions, which in turn often exacerbates the disease state. ***(Endocrine Reviews 40: 66 – 95, 2019)***

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Life on earth evolved under conditions of daily and seasonal rhythmicity imposed by the earth's rotation about its axis and the sun. During the early stages of evolution, earth's hostile environment, particularly the lack of ozone to protect from harmful daytime UV radiation, likely steered toward the selection of a timing system that could predict such daily events. Hence, time is an integral component of our evolution, and mechanisms that can predict time must therefore be deeply implanted into the building blocks of life. The integration of timed schedules into biological systems evolved in the form of molecular clocks that adopted a period of ~24 hours, thus the name "circadian" [derived from the Latin *circa* (about) and *dies* (day)] clock. Circadian clocks are complex oscillating systems that synchronize the organism's physiology with the environment by integrating temporal information about the solar cycle (1). The oscillations generated by circadian clocks are sustained by autonomous feedback loops that are comprised of clock genes.

The Nobel Prize in Physiology or Medicine for 2017 went to chronobiology, which represents an enormous recognition to the field. The prize was awarded to three extraordinary scientists (Jeffrey C. Hall, Michael Rosbash, and Michael W. Young) who dedicated their career to identifying clock genes and understanding the underlying mechanism for the generation and maintenance of circadian rhythms. Notably, the seminal findings of the three Nobel laureates center on the discovery of the *Period* (*Per*) gene and the cyclic expression of its protein (PER) (2–8). Investigations into the rhythmic expression of PER in the fruit fly *Drosophila melanogaster* identified it as an essential component of the transcriptional–translational feedback loop (TTFL) responsible for generating and maintaining circadian oscillations (9). This critical discovery illuminated our understanding

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ESSENTIAL POINTS

- The circadian system is integrated into all forms of life, as evident through its role in driving physiological rhythms and synchronizing them to environmental cycles, and maintaining biological stability
- Clock genes are the interface in the bidirectional communication between the circadian clock and central/peripheral physiological processes
- Physiological processes are intimately linked with circadian clocks; shift work and jet lag impair circadian clocks and clearly increase the risk for metabolic disorders and other disease states
- Clock genes are further involved in a plethora of extraclock functions; the clock gene *Period* in particular has been implicated in multiple central and peripheral processes, from invertebrates to humans
- Cyclic *Period* gene expression essentially implies a tissue- and organ-specific regulation of rhythmic BMAL1-dependent gene expression
- Animal research remains invaluable for proof-of-concept and mechanism-of-action studies related to chronobiological questions on human health

of how organisms, from unicellular prokaryotes to humans, predict and adapt to daily rhythms. This review focuses on the integration of the circadian clock in biological rhythms and with respect to the non-oscillatory function of clock genes, particularly *Period*, on the coupling of the circadian clock to physiology.

PERIOD Within the Central Circadian Network

The circadian clockwork

In mammals, the circadian system is divided into two types of circadian clocks: (1) the central pacemaker and (2) peripheral oscillators, both of which are interconnected via humoral and neuronal networks (10). Biological clocks are comprised of three principal components: an environmental input, a molecular timekeeping mechanism (oscillator), and physiological/behavioral outputs. Input refers to cues present in the environment that provide temporal information to the clock, such as light under alternating light/dark (LD) cycles and food in response to feeding. These time cues are commonly referred to as *Zeitgebers* (German for "time givers") (11). The molecular core oscillator consists of interlocked TTFLs that are autonomous and self-regulating. Figure 1 illustrates a simplified schematic of mammalian TTFLs. Briefly, in the central TTFL, the basic helix–loop–helix transcription factors ARNTL, also known as BMAL1 brain and muscle Arnt-like protein-1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK), dimerize and bind to E-boxes on promoters of *Cryptochrome* [(*Cry*)*1*/*2*] and *Per* (*Per1*–*3*) genes (12). PER and CRY proteins dimerize in the cytoplasm before translocating into the nucleus, where they bind to and inhibit E-box transactivation by BMAL1:CLOCK, thus suppressing their own gene expression (Fig. 1). This molecular oscillation is self-sustained and runs with a remarkably precise period of ~24 hours (13). Temporal information provided by *Zeitgebers* is integrated into the molecular timekeeping mechanism, and, as a result, cellular clocks and downstream clock-dependent physiology are aligned with external time. The circadian timekeeping mechanism is dependent on the rhythmic transcription of clock genes, the protein products of which in turn control output genes that affect physiological, metabolic, and behavioral rhythms (14), hence the coupling of physiological processes known as the output to upstream circadian oscillators. As such, the molecular clockwork modulates physiological processes and ensures their optimal coordination to a cycling environment, thus maximizing resource efficiency and enhancing the chance of survival.

The discovery of the *Period* genes

The clock gene *Period*, a core component of the central TTFL, was first discovered in *D. melanogaster*. Three original mutations of *period* (*per*) were found to affect the circadian locomotor activity of adult flies in an observable way: per^{S} shortened activity, per^{L1} lengthened activity, and per^{O1} abolished rhythmic locomotion altogether (15). Further cloning and immunohistochemical analyses demonstrated a broad expression of *per* in numerous tissues throughout the adult *Drosophila* body (6, 16–19).

The function of *per* as a negative-feedback modulator of circadian clocks was first proposed by Hardin *et al.* (3) in 1990. Prior to their investigation, it had been established that *Drosophila* PER proteins oscillate following a circadian rhythm, and that this expression is affected by *per* gene mutations. Hardin *et al.* analyzed *per* mRNA levels in *Drosophila* brains under 12/12-hour LD cycles and in the absence of time cues, in this case constant darkness (DD). The study shows that *per* mRNA oscillates in a circadian fashion. It was then proposed that cycling *per* mRNA is likely the mechanism behind the rhythmicity of PER proteins.

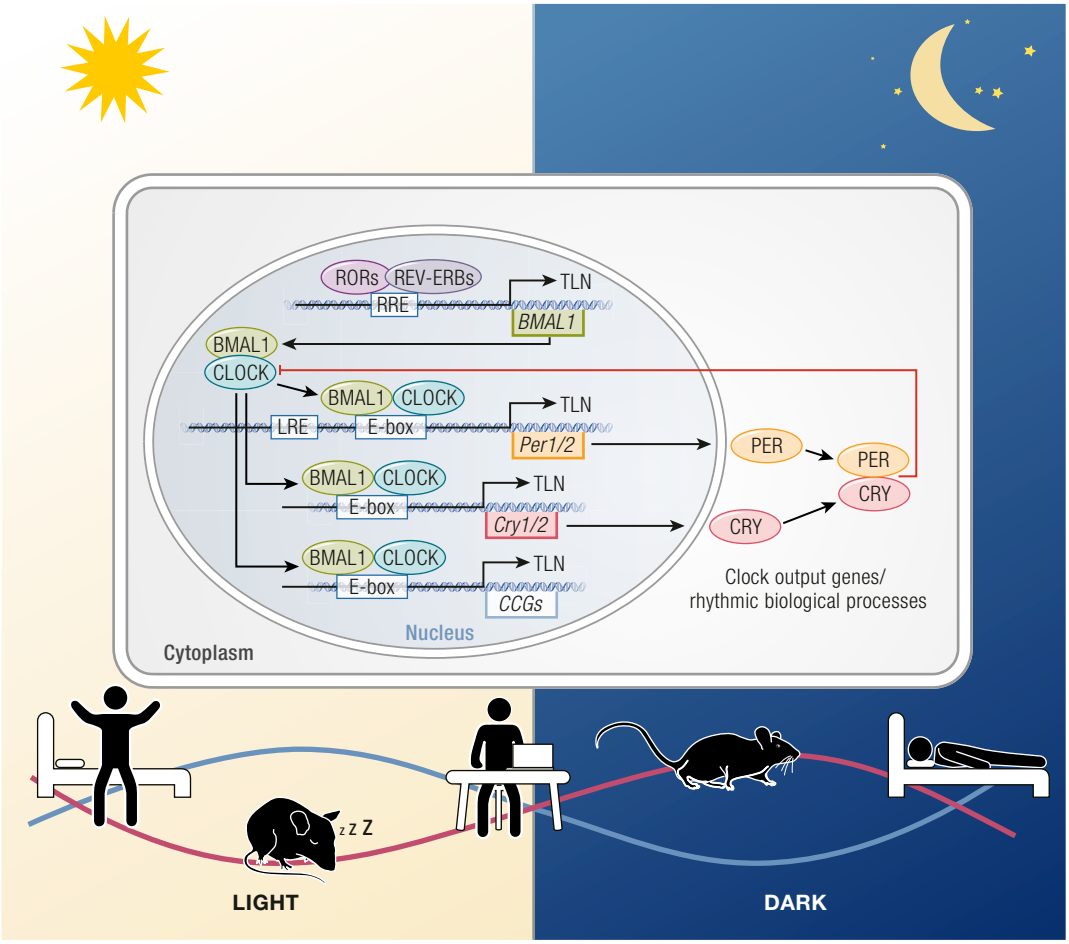


Figure 1. Schematic of the core TTFL of the mammalian circadian oscillator. The positive elements, *BMAL1* and *CLOCK*, heterodimerize in the nucleus and bind to E-boxes in the promoter region of *Cry*s (*Cry1* and *Cry2*), *Per*s (*Per1*–*3*), and clock-controlled genes (CCGs) activating their expression. The *PER:CRY* complex binds to *BMAL1*:*CLOCK* to inhibit *Per*/*Cry* transcription. *REV-ERB*/*ROR* additionally modulates *Bmal1* transcription by binding to the ROR response element (RRE) region of the respective promoter. Transcriptional activation of *Per* genes is also under the influence of light and involves the light-responsive element (LRE, also known as CRE). [© 2019 Illustration Presentation ENDOCRINE SOCIETY].

Rescue experiments performed with per^{O1} mutant flies suggested that PER protein levels may similarly affect the cycling of *per* mRNA, leading Hardin *et al.* (3) to speculate about the existence of a feedback loop involving *per* and its protein product as a central feature of *Drosophila* circadian clocks. Later studies such as that performed by Zeng *et al.* (20) confirmed this assumption by overexpressing PER in photoreceptor cells in *Drosophila* and observing the suppression of cycling *per* RNA. It was concluded that the PER protein is a negative regulator of its own expression and that this feedback occurs at the cellular level in flies.

Interest into *per* was further intensified by the discovery of a mouse and human *PER* ortholog by two independent sources: Sun *et al.* (21), who named them *RIGUI* after the ancient Chinese sundial, and Tei *et al.* (22), who named them *mPer* (mouse) and *hPER* (human) due to their protein sequence similarities with *Drosophila per*. The functional homology between mammalian and insect *Per*s was confirmed by Shigeyoshi *et al.* (23) who observed that introducing *mPer* into arrhythmic per^{O1} mutant flies restored behavioral rhythmicity. Further investigation led to the discovery and isolation of other mammalian paralogs of *per*. As such, the naming convention "*mPer1*" was adopted to refer to the original mouse ortholog, whereas "*mPer2*" and "*mPer3*" were attributed to its later identified paralogs, the functions of which are discussed later in the review.

A marriage between light and PERIOD proteins

Following the discovery of clock gene components in the mammalian circadian system, research focused on investigating how light, the strongest *Zeitgeber*, entrains the central mammalian pacemaker. Although oscillatory patterns of clock gene expression exist in circadian clocks across a wide variety of species, the phasing and mechanisms of clock resetting by light differ. To further study the effects of light exposure on the expression of *Per1* in the central pacemaker of mice, Shigeyoshi *et al.* exposed mice under DD conditions to a 30-minute light pulse at different phases of their sleep–wake cycle. Their study produced three major observations regarding *Per1*: first, that rhythmic *Per1* expression is rapidly reset by a light pulse. This reset is characterized by an initial photoresponsive spike in which *Per1* mRNA transcripts increase by as much as fivefold to eightfold in the suprachiasmatic nucleus (SCN). Second, the magnitude of *Per1* induction shows a positive correlation

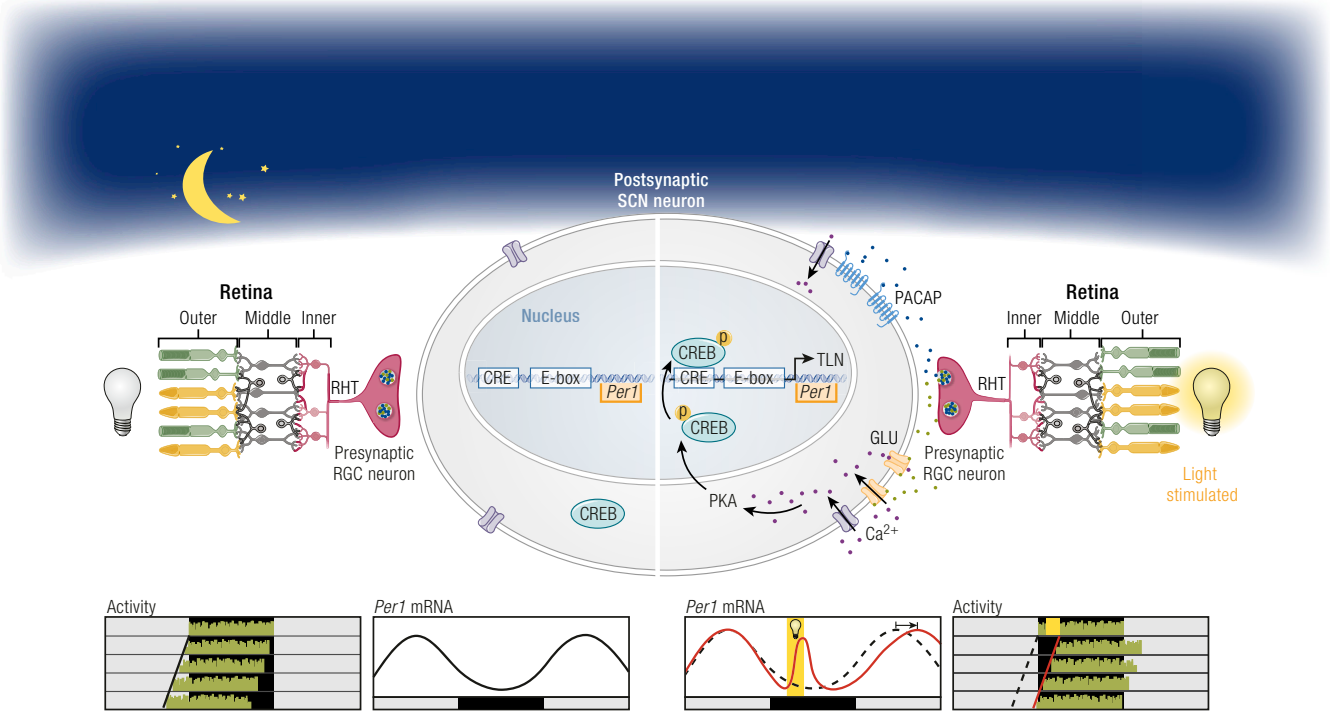
with the extent of behavioral rhythm resetting. Finally, *in situ* hybridization experiments of *Per1* in the SCN revealed a differential expression pattern of *Per1* within the SCN, with *Per1* expression levels generally low in the dorsal, ventral, and medial portions of the SCN. After light pulse administration, Shigeyoshi *et al.* observed an induction of *Per1* expression that first started in the ventral SCN before spreading more dorsally. Such findings were the first to show the ventral SCN as the site of the light input into the SCN. Taken together, *Per1* is a clockwork component that also functions as a photoresponsive immediate early gene whose light induction correlates with behavioral phase resetting and clock gene rhythm resetting of the mammalian central pacemaker within the SCN. A schematic of light-induced resetting of the central clock is shown in Fig. 2.

Additionally, other studies sought to determine the effects of light exposure on the *Period* paralog *Per2*, which was isolated and identified by Albrecht *et al.* (24). Both PER proteins (PER1 and PER2) contain two PER-ARNT-SIM (PAS) domains (PAS-A and PAS-B) (25). Structurally, PER2 differs from PER1 by a single amino acid exchange within its PAS domains, a feature to which many of the nonredundant functions of PER2 have been attributed (25). Similar to *Per1*, *Per2* mRNA expression oscillates rhythmically in the SCN (Table 1) (26–42), but with a 4-hour phase delay compared with the *Per1* rhythm. Also similar to *Per1*, *Per2* expression rhythms are entrained (phase aligned or synchronized) to the external LD cycle and *Per2* transcription can be acutely induced by light; however, the extent of this response depends on the circadian time of light exposure. Albrecht *et al.* and others (43–45) showed that although *Per1* transcription is light-responsive throughout the (subjective) night, photic induction of *Per2* is high during the beginning and low toward the end of the night. Therefore, to further elaborate on the role of *Per1* and *Per2* in light-induced clock resetting, the activity of mice carrying *Per1* or *Per2* mutations was analyzed in response to light pulses applied at the early or late subjective night. A light pulse in the early night induces phase delays in sleep–wake rhythms in wild-type (WT) and *Per1* mutant mice, whereas light pulses at late night trigger phase advances only in WT mice but not in *Per2* mutant mice. Taken together, *Per1* and *Per2* genes play a pivotal role in the photic resetting of the circadian clock (45).

To assess additional functions of *Per2* within the mouse circadian system, Zheng *et al.* (46) generated and identified a *Per2* loss-of-function mutation. This mutation ($Per2^{Brdm1}$) is characterized by a two-exon deletion encoding for the region of PER2, which is most conserved between mouse and *Drosophila per*. Homozygous $Per2^{Brdm1}$ mice show shortened circadian periods of locomotor activity, followed by a loss of circadian rhythmicity (arrhythmicity) in the absence of time cues (*i.e.*, under DD conditions), under which conditions WT mice free-run with incredible accuracy and steadiness with a cycle time that approximates the period length of the earth's rotation about its axis (24 hours; hence the term *circa*-dian). $Per2^{Brdm1}$ mice display diminished cycling transcript levels of both *Per1* and *Per2* in the SCN, which was unexpected given the assumed role of *Per* genes as negative regulators of transcription in the mammalian TTFL. Considering that a truncated form of *Per2* is still expressed in the $Per2^{Brdm1}$ mice, these studies may suggest that PER2 functions as a positive regulator of *Per1* expression, or alternatively the existence of an autoregulation and cross-regulation between the two *Per* genes in an independent molecular cycle.

Similarly, mice homozygous for loss-of-function mutations in *Per1* show robust circadian activity cycles in the presence of time cues (light; under LD conditions) and a shortened circadian period (~1 hour) under DD conditions (30). In contrast to $Per2^{Brdm1}$ mutants, $Per1^{Brdm1}$ animals display persistent robust rhythmicity of (non–protein coding) *Per1* and *Per2* transcripts in DD, comparable to WT controls (30). Thus, PER1 is not essential to maintain its own circadian expression. Taken together, the PER1 protein appears to be dispensable with regard to maintaining circadian behavior, whereas PER2 is involved in regulating circadian gene expression via transcriptional control (30, 47). More specifically, PER2 exerts a positive regulation on mammalian clock gene expression because loss of PER2 function significantly reduces peak expression of *Per1*, *Per2*, and *Cry1* (46, 48). Although *Per1* and *Per2* single-mutant mice are rhythmic under both LD and constant conditions, *Per1/2* double-mutant mice are arrhythmic in their sleep–wake cycles and core clock and clock-regulated gene expression profiles. Collectively, this suggests that *Per1* and *Per2* are important for circadian clock control (30). Furthermore, the immediate and complete loss of rhythm phenotype in *Per1*/*Per2* double-mutant mice in constant conditions (DD) suggests that PER1 and PER2 cooperate in the core clock mechanism. The normal activity pattern of the *Per1*/*Per2* double mutants under LD conditions raises the possibility that there is some residual clock function. However, this possibility was dismissed with the discovery that a light pulse could not reestablish a circadian rhythm in these mice (30) as is the case for the single mutants (46), which is consistent with a complete loss of a functional clock. An alternative explanation is that the behavioral adaptation to LD maybe due to a masking effect of the light (through direct photic inhibition of locomotor activity). In case of a true loss of clock control the animals are expected to respond passively to external cues as observed in double and single *Per* mutants (30). Thus, *Per1* and *Per2* couple the animal's rhythmicity to an internal clock to be influenced, but not driven, by external cues.

Figure 2. Light-induced resetting of the central clock. Left, Unstimulated retinorecipient SCN neuron. Right, Light-stimulated SCN postsynaptic neuron. Light at night activates melanopsin-positive intrinsically photosensitive retinal ganglion cells (ipRGCs) of the RGC layer (inner layer; red), which also receives photic information from the rods and cones (outer layer; green and yellow) through amacrine, bipolar, and horizontal cells (middle layer). Photic input transmission from ipRGCs along the retinohypothalamic tract (RHT) triggers the release of neurotransmitters such as glutamate (Glu) and pituitary adenylate cyclase–activating polypeptide (PACAP) from presynaptic RGC neurons of the retina. Glu and PACAP then bind to their corresponding receptors on the postsynaptic SCN neurons, resulting in membrane depolarization via the influx of Ca^{2+}. Intracellular increase in Ca^{2+} levels activates kinases, including PKA, leading to the phosphorylation of CREB. pCREB, in turn, binds to the *CRE* element of the *Per1* promoter in the nucleus, driving *Per1* expression. The rapid surge in *Per1* expression accompanies a phase delay of locomotor activity rhythms (red line) relative to the activity rhythm of an animal free-running under constant darkness (black line). [© 2019 Illustration Presentation ENDOCRINE SOCIETY].



The second paralog of *Per1* was identified by Takumi *et al.* (44) and was subsequently named *Per3*. PER3 retains the highly conserved PAS domain that is shared among the PER paralogs, with a closer resemblance to that of PER1 than of PER2 (25). Several clusters of homologous sequences are present in the C-terminal region of the PAS domain for all PER proteins, supporting that *Per3* is indeed a member of the mammalian *Per* family. Similar to the other *Per* paralogs, *Per3* is rhythmically expressed in many regions of the brain. In the SCN, its circadian transcript profile closely resembles that of *Per1* and *Per2* with a peak during the subjective day (49) (Table 1). Unlike the other *Per*s, however, *Per3* transcription is nonresponsive to light.

Intriguingly, by evaluating the phase response curve for light on locomotor activity as a reliable predictor for photic entrainment (synchronization) in $Per1^{-/-}$, $Per2^{-/-}$, and $Per3^{-/-}$ mutant mice, Pendergast *et al.* (50) discovered that *Per* mutant mice showed a differential phase shift in their activity in response to the light pulses. Furthermore, *Per1* mutant mice entrain to a wider range of non–24-hour LD cycles (T-cycles) (from 21- to 26-hour cycles) compared with WT, *Per2*, and *Per3* mutants who show much narrower limits of entrainment. Collectively, this suggests a connection between the shape of the photic phase response curve and the plasticity of the circadian clock. Notably, $Per1^{-/-}$ is the only mutant with both significantly larger advance and delay zones compared with WT mice, which explains the ease by which their clock aligns with extreme T-cycles. Although, alterations in the sensitivity to light in *Per* mutants require further investigation, it is accepted that *Per* genes have functionally distinct roles in circadian clock control.

The role of Period in circadian clock resetting

Shigeyoshi *et al.* (51) were the first to report on the spatiotemporal dynamics of *Per1* expression in response to a light pulse. The magnitude of light-induced *Per1* induction was found to be at odds with the relatively weak light-induced phase shift in the organism's sleep–wake rhythm. The authors suggested that cycling *Per1* expression levels in the SCN, and the compartmentalization of *Per1* to the

ventral SCN, shape the light-induced behavioral shifts. The time-of-day–dependent expression levels of *Per1* within the ventral SCN subregion are thought to set the responsiveness of ventral SCN neurons to photic stimuli at the cellular level (51). The role of *Per* in phase resetting is thought to be exerted in a time-of-day–dependent manner. *Per1* and *Per2* mutant mice reset sleep–wake cycles differently depending on the time of nocturnal light exposure (45). A subsequent study by Yan and Silver (52) further elaborated on the differential functions of *Per* in resetting the mammalian clock. The authors investigated the induction and localization of *Per1* and *Per2* in the SCN with focus on the heterogeneity of SCN neurons upon delaying and advancing phase shifts. They showed that the SCN can be divided into two anatomically distinct regions: a ventral region (core), which receives direct retinal input and contains vasoactive intestinal peptide–expressing neurons, and a dorsomedial zone (shell), consisting largely of arginine vasopressin–expressing cells. The time course and the localization of light-induced *Per1* and *Per2* expression differentiate between the ventral and dorsomedial regions of the SCN. Light administration substantially induces *Per1* expression in the core throughout the subjective night, whereas strong light-induced *Per2* expression in this area is only observed after a phase-delaying light pulse. In contrast to the SCN core, light-stimulated *Per* expression in the shell region was found to align with behavioral phase shifts. A phase-advancing light pulse leads to significant *Per1* expression, although substantial induction of *Per2* is only observed in response to a phase-delaying light pulse, and both *Per1* and *Per2* are not induced following a light pulse where no behavioral phase shift is observed. These findings indicate that light-induced *Per1* or *Per2* expression in the SCN core is insufficient to generate behavioral phase shifts. Rather, differential *Per1* and *Per2* gene

Table 1. Evidence for Expression of *Per1–3* in Central and Peripheral Tissues in the Mouse

	Circadian Rhythm of *Period* Genes in Mice						
					Expression Under 12/12 LD		
	Tissue/Organs	Per Genes	Strain	Rhythmic Under Constant Conditions	Peak	Trough	References
Brain	SCN	*Per1*	129/sv, CD-1-ICR, C57BL/6	Yes	ZT8	ZT20	(26–30)
		Per2	129/sv, CD-1-ICR, C57BL/6	Yes	ZT10	ZT22	(26–30)
		Per3	Unknown	Yes	ZT8	ZT0	(29)
	Hippocampus	*Per1*	C3H/He	yes	ZT10	ZT22	(31, 32)
		Per2	C3H/He	Yes	ZT12	ZT0	(31, 32)
Periphery	Adipose tissue	*Per1*	C57BL/6	Yes	ZT12	ZT0	(29, 33)
		Per2	C57BL/6	Yes	ZT14	ZT2	(29, 33, 34)
		Per3	N/A	Yes	ZT12	ZT0	(29)
	Heart	*Per1*	BALB/c, C3H/He	Yes	ZT10	ZT20	(35, 36)
		Per2	BALB/c, C3H/He	Yes	ZT12	ZT0	(35, 36)
		Per3	BALB/c, C3H/He	Yes	ZT12	ZT20	(35, 36)
	Liver	*Per1*	BALB/c, C57BL/6, C3H/He	Yes	ZT10	ZT22	(29, 35–37)
		Per2	BALB/c, C57BL/6, C3H/He	Yes	ZT12	ZT0	(29, 35–37)
		Per3	BALB/c, C3H/He	Yes	ZT10	ZT22	(29, 35, 36)
	Adrenals	*Per1*	C57BL/6, C3H/He	Yes	ZT10	ZT22	(38–40)
		Per2	C57BL/6, 129S2	Yes	ZT12	ZT0	(38, 39, 41)
		Per3	C57BL/6	Yes	ZT12	ZT0	(38)
	Pancreas	*Per1*	C57BL/6	Yes	ZT12	ZT0	(39)
		Per2	C57BL/6	Yes	ZT12	ZT0	(39, 42)

Abbreviations: N/A, not available; ZT, *Zeitgeber* time.

expression in the dorsomedial SCN may be implicated in regulating circadian clock resetting, specifically the directionality of phase shifts (delays or advances) (52). The precise molecular mechanism of how *Per* alters SCN neuronal activity remains unknown.

Kuhlman *et al.* (53) identified a possible molecular pathway of light-induced phase resetting, where light elicits a transient neural response in the SCN core characterized by a change in spike frequency, activation of excitatory receptors, and an increase in intracellular Ca^{2+} concentrations followed by the induction of immediate early genes, including *Per1*. The phase resetting action of light begins within 3 to 5 hours after induction. This delay was attributed to a long-lasting reduction in K^+ current resulting in a consolidated phase shift in electrical neuronal activity, molecular oscillations, and downstream coupled overt rhythms. Notably, Kuhlman *et al.*'s study was restricted to phase-advancing light pulses only. Similarly, Ding *et al.* (54) investigated this differential resetting by determining signaling elements that contributed to phase delays. They found that activators of intracellular Ca^{+2} channel ryanodine receptors induce light pulse–like phase delays of ~3 hours. From this they determined that potential mechanisms of clock resetting specific for phase delays in the SCN may involve the release of intracellular Ca^{2+} through ryanodine receptors, although this component of phase resetting in the central pacemaker still requires further investigation.

Much of the research performed on resetting of the central pacemaker focuses on the phase-shifting properties of light at night, which is also thought to be the dominant regulator of circadian entrainment in mammals (54). Challet *et al.* (55) examined the effects of light at day (LAD) in the context of *Per* expression in the SCN of mice. Short exposure (30 minutes) to LAD resulted in *Per1* and *Per2* induction in the SCN, which could be extended with a prolonged LAD (up to 3 hours). Functionally speaking, the sensitivity of *Per* in the mammalian central pacemaker to changes in lighting conditions may be important for improving the accuracy of synchronization between internal circadian and geophysical time.

Central extra-clock function of PERIOD within the limbic system

The limbic system, an anatomical entity and physiological concept, is a collection of functionally and anatomically interconnected nuclei and cortical structures found in the telencephalon and diencephalon (56). Although these nuclei have different overall roles, they commonly function toward controlling self-preservation and species preservation [reviewed in Ref. (57)]. Hence, they regulate autonomic and endocrine functions, particularly in response to emotional stimuli (58–60). The brain areas typically included in the limbic system are comprised of subcortical structures and the cerebral cortex (61). An example of the latter is the hippocampus, which has (1) paracrine and autocrine functions, such as estrogen synthesis and the differential regulation of hippocampal estrogen receptors (62), and (2) endocrine functions, including control of the hypothalamus–pituitary–adrenal (HPA) axis (63), and commonly is known for its role in (3) memory processing (acquisition, consolidation, and retrieval) (64–66).

Many biochemical processes in the hippocampus, such as protein and neurotransmitter synthesis, synaptic excitability, and neurohormone release, exhibit circadian oscillations (67, 68). Thus, the circadian regulation of local biochemical processes in the hippocampus is likely the mechanism by which the circadian system imposes a temporal regulation on diverse biological functions. Importantly, an organism's ability to process long-term memories (LTMs) varies across a 24-hour day with peaks in memory performance confined to specific time windows within the organism's active phase. To identify the mechanism by which temporal information about daytime structures the efficiency of memory processing or any of the many other functions of the hippocampus, an in-depth understanding of how the circadian clock is integrated into the processing of hippocampus-dependent events is key. Although it seems clear that the hippocampus is a rhythmic structure, particularly at the cell signaling level (69–75), the mechanism by which the circadian clock disseminates temporal information to gate or drive hippocampal molecular rhythms remains under investigation.

It is suggested that the hippocampus operates, in parallel with the amygdala, to modulate body physiology in response to cognitive stimuli, a process known as cognitive sensing (76, 77). Hippocampal outputs are predominantly inhibitory on downstream neuroendocrine activity; increased synaptic efficacy in the hippocampus [*e.g.*, long-term potentiation (LTP)] could facilitate throughput inhibition. One of many examples is its inhibitory action on the HPA axis (78, 79). This is particularly interesting because a previous study reports in *Per1*-deficient mice that adrenal glucocorticoid (GC) levels are arrhythmic and elevated. Hence, changes to clock-controlled hippocampal signaling events may influence the downstream neuroendocrine code. The latter is known to feedback to the hippocampus and modulate hippocampal activity, particularly memory processing (80, 81). In this aspect, we next discuss recent findings on *Per*-dependent regulation of hippocampal signaling and function.

In the mouse hippocampus, long-term synaptic changes are coupled to *de novo* gene expression and posttranslational modifications (82–84), and they rely on intact cAMP/protein kinase A (PKA)/protein kinase C/cAMP response element binding protein (CREB)/MAPK signaling (82, 85, 86), including chromatin remodeling (87–93). Cellular and molecular dynamics in the hippocampus, particularly those

relevant for LTM formation, are molded by time of day (94), which supports an interaction between the circadian system and memory. *Per1* and *Per2* being rhythmically expressed in the mouse hippocampus (31, 95–98), and shown to modulate behavioral sensitization (99), implies a regulatory role for the clock gene proteins PER1 and PER2 in the temporal modulation of hippocampal function (*e.g.*, in learning and memory).

Circadian core clock components are rhythmically expressed in the hippocampus of $Per1^{-/-}$ mice, yet the phases of clock gene expression rhythms in $Per1^{-/-}$ mice are shifted compared with WT mice, despite that the SCN clock of $Per1^{-/-}$ mice is properly phased to ambient lighting conditions (31). $Per1^{-/-}$ mice are rhythmic under both diurnal and constant conditions similar to control littermates ($Per1^{+/+}$), suggesting that PER1 may have a specific local role in modulating hippocampal physiology (74, 75, 100). This is supported by *in vitro* studies showing that cAMP/PKA signaling to the memory-dependent transcription factor CREB is impaired in primary hippocampal neurons derived from $Per1^{-/-}$ mice (75). This may explain why the diurnal rhythm of hippocampal CREB phosphorylation (71) is lost in $Per1^{-/-}$ mice (74). Notably, the rhythmic phosphorylation of both MAPK and CREB in the mouse hippocampus is important for the maintenance of LTM (71, 72).

Long-lasting changes in synaptic plasticity known as LTP are the cellular correlate for LTM (84, 86). The magnitude of LTP at perforant path–granule cell synapses in the dentate gyrus is compromised in $Per1^{-/-}$ mice, whereas basic properties of synaptic transmission appear normal, indicating that functional deficits are not likely due to alterations in network excitability (75). The recorded reduction in LTP amplitude observed in $Per1^{-/-}$ mice may suggest a specific role for PER1 in the reinforcement and consolidation of spatial memories.

Long-term memory formation depends on different signaling cascades, many of which converge to activate the transcription factor CREB to initiate LTM-dependent gene expression (101–103). The silencing of one or several of these pathways will likely alter learning-induced dynamics in CREB activation, and consequently affect LTM formation. Importantly, note that although $Per1^{-/-}$ mice show a reduction in the amplitude of *in vivo* LTP, they do acquire LTM. However, in contrast to $Per1^{+/+}$ mice, day/night differences in memory performance are absent, at least when comparing the *Zeitgeber* time 02 and 14 time points. This may be linked to the absence of day/night variations in phosphorylated CREB (pCREB) levels in $Per1^{-/-}$ mice [reviewed in Ref. (104)].

It is tempting to assume that the mechanism by which temporal information is integrated into LTM processing involves a rhythmic interference of cyclic clock proteins such as PER1 with memory-relevant molecular signaling events. Clockwork components may have non–clock-related functions. *Per2*, for example, is constitutively expressed in the dentate gyrus of the hippocampus (105), and thus it cannot possibly convey temporal information by rhythmically interfering with local events. Therefore, deficits in long-term trace fear memory observed in *Per2* mutant mice (96) reflect an extra-clock function of PER2. The regulation of hippocampal neurogenesis and, hence, neurogenesis-dependent memory formation [reviewed in Ref. (106)] is another intriguing example of an extra-clock function for the core clockwork component PER2. The expression of rhythmic clock genes in the hippocampus suggests a functional role for the hippocampal circadian oscillator in memory processing and beyond, as highlighted by several reports [reviewed in Refs. (72, 94, 105)]. Interestingly, studies investigating the role of clock genes in hippocampal functions suggest that different clock genes are involved in modulating different types of hippocampus-dependent behaviors (31, 107, 108). The herein reported role of *Per* genes in hippocampal signaling and how it translates to LTM processes is one example and supports further investigations into more downstream hippocampus-regulated endocrine functions.

There is compelling evidence to suggest that hormones as well as metabolic signals can modulate circadian oscillations of clock gene expression in the brain and the periphery. The pineal hormone melatonin, for example, modulates the rhythm of *Per1* expression in the pituitary gland, striatum, and adrenal cortex (109–111). Furthermore, adrenal GCs were shown to modulate the rhythm of expression of PER2 in the oval nucleus of the bed nucleus of the stria terminalis and the central nucleus of the amygdala (112, 113). This suggest that clock genes, being an integral component of memory processing and limbic function, also serve to communicate peripheral endocrine functions to central processes.

"Clock genes in general and period genes in particular are rhythmically expressed in most peripheral tissues."

PERIOD Within the Peripheral Circadian Network

General statements

As briefly introduced, the circadian system is composed of two categories of circadian clocks: the central pacemaker in the SCN, and peripheral oscillators distributed throughout the body. The latter time the rhythmic expression of tissue- and organ-specific genes (114, 115), therefore emphasizing the importance of temporal coordination of various biological processes (116). Oscillations of physiological processes in peripheral tissues are shown in Fig. 3. For example, in diurnal mammals, insulin responses (resulting in glucose disposal) are highest during daytime, when carbohydrate uptake peaks due to SCN-regulated daytime activity and food intake. Similarly, glucagon secretion peaks at night so that stored glycogen may

efficiently be converted to glucose to counteract decreased serum glucose levels during fasting (117). This coordination of central and peripheral rhythms is vital to efficiently maintain resources and metabolic homeostasis.

Clock genes in general and period genes in particular are rhythmically expressed in most peripheral tissues (36, 118) (Table 1). However, there are large organ-to-organ (or tissue-to-tissue) variations in the phase and amplitude of clock gene expression rhythms, the characterization of which is primarily based on rodent models, particularly mice. Recently, however, a shift toward assessing human tissue clock gene expression rhythms has been initiated (119–121) (Table 2) (120, 122–134). This section focuses on rodent clock gene expression rhythms across peripheral organs and tissues, addressing how they are linked to tissue-specific functions.

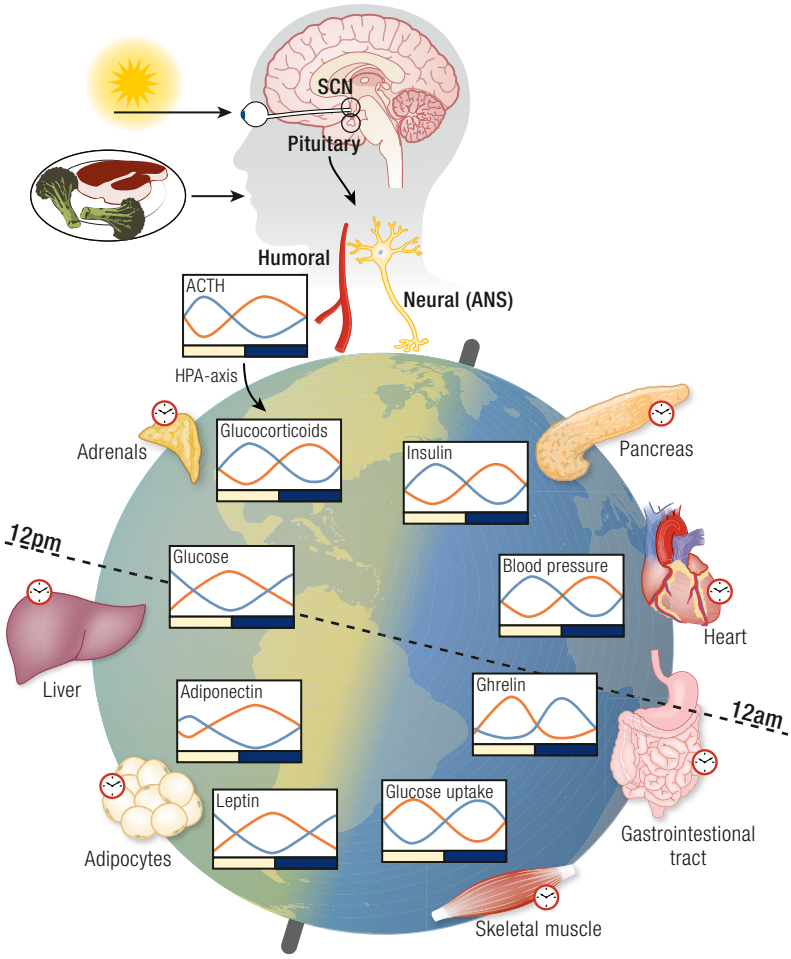


Figure 3. Central-to-peripheral circadian clock coupling. Cycles of day and night resulting from the earth's rotation about its axis synchronize the central SCN clock. The SCN clock disseminates temporal information about daytime to peripheral clocks via neural and hormonal pathways to synchronize and coordinate physiological rhythms in the periphery, thus aligning peripheral physiological rhythms to geophysical time. All peripheral organs house circadian oscillators, many of which can also be influenced by the timing of food intake. Although diurnal (blue) and nocturnal (orange) species generally share the same clockwork components, their physiological and behavioral rhythms are antiphasic. [© 2019 Illustration Presentation ENDOCRINE SOCIETY].

In the context of circadian rhythms and the endocrine system, it is important to understand the degree of coupling between the central pacemaker and peripheral oscillators in the regulation of organ-specific functions. One method of investigation involves the use of organ- or tissue-specific knockout models of clock genes (135). Several different aspects have to be considered to address the connection between expression of tissue-specific genes and circadian functions. First, we consider the different cell types within an organ, which obviously have distinct functions and can be defined by the expression of specific sets of genes. Second, we consider the degree by which a tissue/organ-specific circadian rhythm is intrinsically regulated by a local oscillator or guided by the central pacemaker in the SCN. This poses the question of whether clock genes can be considered as housekeeping genes, especially because most cells of the body are capable of sustaining circadian rhythms driven by autonomous TTFLs comprised of clock genes. This aspect of tissue- and time-specific expression of genes has not been investigated in detail until recently (136). Tissue-specific gene interactions with clock genes could be investigated using a bottom-up (from *in vitro* cell culture, *ex vivo* organotypic cultures to *in vivo* experiments) or a top-down approach by inactivating a specific gene of interest within a particular cell type to assess its function at the organismal level. Both strategies share potential drawbacks in determining the functional role of a gene. For instance, specific cells can react differently, depending on whether other cell types are present in the culture, and *in vivo* gene inactivation may yield compensatory phenotypes due to genetic nonallelic or epigenetic compensation mechanisms.

Peripheral circadian clocks

Liver

The liver plays a pivotal role in energy metabolism by maintaining glucose homeostasis through a tight regulation of catabolic and anabolic processing of glucose in response to energy demands (137). Diurnal and circadian variations of liver parameters have been known for a very long time (138). Unlike the SCN, the liver is dependent on systemic nonphotic time cues for its entrainment such as GCs or food intake–associated signals (139). Although not being directly regulated by light, it receives photic information through the SCN (140) (Fig. 3). Numerous studies have demonstrated the existence of a hepatic clock, with clock gene expression (*Per1*, *Per2*, *Per3*, *Bmal1*, *Cry1*, and *Cry2*) cycling in a circadian fashion (114). Besides rhythmic clock gene expression, ~15% of liver transcripts show a circadian rhythm in DD (141) and are linked to carbohydrate and energy metabolism, xenobiotic detoxification, and lipid homeostasis (142). Notably, these liver-specific rhythms are downstream outputs of

Table 2. Evidence for Expression of *Per1–3* in Central and Peripheral Tissues in Humans

	Circadian Rhythm of *Period* Genes in Humans				
	Tissue/Organs	***Per* Genes**	**Rhythmicity**	**Peak**	**References**
Brain	Pineal gland	*Per1*	Yes	During day	(122–124)
		Per2	No	N/A	(123)
	Hippocampus	*Per1*	Yes	Early morning	(125)
		Per2	Yes	During day	(125)
		Per3	Yes	During midday	(125)
Periphery	Leukocytes/blood mononuclear cells	*Per1*	Yes	During early morning	(126–128)
		Per2	Yes	During early morning	(126, 127)
		Per3	Yes	During day	(126, 129, 130)
	Bone marrow	*Per1*	Yes	During morning	(131)
		Per2	Yes	During morning	(131)
	Heart	*Per1*	Yes	During early morning	(120)
		Per2	Yes	During morning	(120)
	Adipose tissue	*Per1*	Yes	During morning	(132, 133)
		Per2	Yes	Meal time dependent	(129, 132, 133)
		Per3	Yes	During morning	(129, 132, 133)
	Skeletal muscle	*Per1*	Yes	During morning	(134)
		Per2	Yes	During morning	(134)

Abbreviation: N/A, not available.

the local hepatic oscillator because the knockdown of the core clock gene *Bmal1* in the liver was shown to interfere with hepatic function, rendering the aforementioned physiological processes arrhythmic (143).

The study by Ramanathan *et al.* (144) looked into cell-specific effects of downregulating *Per* genes using lentiviral short hairpin RNA vectors for *Per1*, *Per2*, and *Per3*. The results show that knockdown of either *Per1*, *Per2*, or *Per3* results in short-period, low-amplitude rhythms of cycling clock genes within hepatocytes. Double and triple knockdowns of *Per1/2* and *Per1/2/3*, respectively, result in complete arrhythmicity, similarly to the results reported by Kornmann *et al.* (142) using liver-specific REVERBα-overexpressing mice. In addition to these findings, a recent and very intriguing report showed that in *Per*-null mice, polyploidization of hepatocytes is markedly accelerated, possibly due to an impaired MAPK phosphatase–mediated alteration in ERK1/2 activity. These data clearly support the notion that *Per* genes are not only responsible for regulating period length and amplitude of hepatocyte clocks and metabolic activity, but also for hepatocyte turnover and self-renewal, with *Per* deficiency leading to a massive accumulation of polyploid cells and potentially favoring malignant transformation (144). In line with this, mice with a deletion of the *Per2* gene (46) display the three cancer hallmarks, including uncontrolled cell proliferation, genomic instability, and tumor-promoting inflammation. This is reflected by profound alterations in hepatic oncogenic gene expression, including the avian myelocytomatosis virus oncogene cellular homolog (*c-Myc*), Wee1 tyrosine kinase, G2/mitotic-specific cyclin-B1 (*Ccnb1*), and Kirsten rat sarcoma GTPase (*K-ras*), as well as increased inflammation with high IL-6 levels in the absence of any carcinogen exposure. These findings predict that functional mutations in the *Per2* gene increase the chance to develop liver carcinoma. In fact, *Per2* mutant mice are nearly four times more likely to develop cancer as compared with controls (145). Alternatively, overexpressing *Per2* significantly suppresses tumor growth *in vivo* by improving tumor cell adhesion and suppressing cell migratory activity (146). This newly discovered role of the *Period* gene adds to its known circadian function with respect to hormone production, development, regeneration, and healthy aging of liver tissue.

In recent times, the description of the circadian liver proteome (147) gave the field a fresh look and has

since been extended and advanced by analyses of clock-dependent and clock-independent liver proteomes (148). These studies have been further complemented by the evaluation of posttranscriptional mechanisms (149) and rhythmic degradation of protein products in the liver (150). In this context, it has also been shown that the mitochondrial proteome of the liver depends on the presence of the PER proteins within the cell nuclei of hepatocytes (151). More recent in-depth quantitative mass spectrometric analysis of the liver nuclear proteome (152) and the liver phosphoproteome revealed that ~500 of the 5000 nuclear proteins enter and leave the hepatocyte nuclei in a circadian manner and 25% of the protein phospho-sites oscillate diurnally. Moreover, the lysine "acylome" of liver proteins, the group of proteins containing acetylated lysine, which is very relevant for the orchestration of metabolic activity of the liver, are under diurnal (and feeding) control (153).

Another interesting question to raise is whether synchronizing factors rhythmically produced by the central circadian clock can target liver function. This idea originates from a classical experiment showing that the reintroduction of the SCN, housed within a capsule permeable to molecules up to the size of proteins, but not nerve fibers, can reinstate sleep homeostasis (154). Follow-up experiments confirmed that such SCN-specific factors (peptides and proteins) exist and are indeed responsible for internal synchronization (155, 156). Alternatively, adropin, a peptide hormone produced in the liver and secreted in a diurnal manner, was shown to act on the brain (157). This would add another level of complexity to the mutual influence of peripheral circadian outputs on central clocks. Mining the data sets originating from the mass spectrometric studies mentioned above may allow the discovery of new peripheral tissue-specific rhythmic peptides or proteins that couple peripheral clocks to the central biological clock.

Adrenals and the HPA axis

The adrenals are the effector tissue of the HPA axis and are central in the regulation of multiple physiological functions, including gluconeogenesis, lipid metabolism, immunity, stress response, and reproduction (158). Most prominently and well studied, however, is the adrenals' role in synchronizing behavior and physiology *via* the rhythmic secretion of GCs (159). Extensive studies on the mechanisms driving 24-hour GC rhythms revealed that the adrenal houses a circadian oscillator (40, 160). Robust rhythmic expression of canonical clock genes, including all three *Per* paralogs, was found within the adrenal cortex (and to a lesser extent in the adrenal medulla) of rodents (40, 160). Consistent with most other tissues studied and across experimental models, circadian rhythms in the expression of adrenal clock genes could also be verified in nonhuman primates (161), which similarly suggests the presence of an adrenal circadian oscillator. Additionally, a transcriptional circadian profiling study of the adrenal led to the discovery that adrenal genes involved in endocrine functions, including steroid and cholesterol biosynthesis, and catecholamine metabolism are under circadian control (162). A recent study emphasized the importance of the adrenal clock in the regulation of daily rhythmic release of GCs, showing that when central and peripheral rhythms are uncoupled by daytime-restricted feeding in mice, adrenal GC content and steroidogenic acute regulatory (StAR) protein levels are phase shifted. This is consistent with a shifted rhythm in clock gene expression in the adrenal (163). Notably, restricted feeding leads to two distinct peaks in plasma GC levels: an early peak that corresponds to a shift in adrenal steroidogenesis, and a later peak that is in phase with the organism's sleep–wake cycle, and thus represents an SCN-dependent rhythm. These findings indicate that both SCN-driven autonomic innervation and the adrenal clock are crucial in driving the daily GC rhythm and its stabilization on a daily basis (163).

Although the SCN is required to generate the diurnal pattern of the HPA axis (164), the role of *Per* genes in this process is not well characterized. Yang *et al.* (165) assessed the circadian rhythm of the HPA axis in $Per2^{+/+}$ and $Per2^{-/-}$ mice. The lack of corticosterone rhythm in $Per2^{-/-}$ mice suggested a defect in either corticosterone production or generation of HPA rhythm. Although $Per2^{-/-}$ mice lack a recognizable corticosterone rhythm, they can produce corticosterone in response to stress. This suggests that *Per2* may be important for generating the ACTH rhythm and/or the adrenal cortical rhythm.

Although the central (hypothalamic–pituitary) activity of the HPA axis may play a part in the phase adjustment of hormone production, it may be less critical for generating the daily rhythms of GC secretion. Ishida *et al.* (166) demonstrated that light exposure during the night leads to a rapid induction of *Per1* in the adrenals and a subsequent increase in plasma corticosterone (the primary GC in rodents) independently of the adrenocorticotrophic hormone (ACTH). Interestingly, denervation of the adrenals abolishes photic induction of *Per1* in the adrenal cortex, which is accompanied by a reduction in GC responses. This suggests that sympathetic innervation rather than hypothalamic–pituitary activation is the predominant route by which the SCN relays photic information from the environment to the adrenals. In conjunction with previous observations, Ishida *et al.*'s study provides strong evidence for the presence of a separate peripheral clock within the adrenals and also implies a potential role of *Per1* in light-evoked

physiological changes in GC signaling. Later investigations by Oster *et al.* (38) sought to confirm the presence and characterized the function of this adrenal clock. The group confirmed the presence of an adrenal circadian oscillator and reported robust rhythmic expression of canonical clock genes, including all three *Per* paralogs within the adrenal cortex. They also observed that circadian rhythms of the ACTH, corticosterone, and clock gene expression are abolished in *Per2/Cry1* mutant mice, which is consistent with their previous observations of disrupted locomotor and feeding activity (167). Examination of adrenal slice cultures led to the discovery that the adrenal cortex shows a gated sensitivity to ACTH; that is, ACTH stimulation triggers corticosterone release in a temporally controlled fashion in the absence of SCN input (38). Clock-deficient adrenals lack this sensitivity, which indicates that the responsiveness to ACTH relies on a functional adrenal clock. To confirm this mechanism *in vivo*, Oster *et al.* (38) transplanted WT adrenals into clock-deficient *Per2/Cry1* double-mutant hosts and into WT hosts lacking adrenals. Transplantation of WT adrenals into mutant hosts restored corticosterone rhythmicity, whereas mutant adrenal transplantation into WT hosts dampened corticosterone expression. Such observations illustrate the mechanism of the gated adrenal response to ACTH, wherein the constant (*i.e.*, rhythm-ablated) ACTH signaling in mutant hosts is translated by the WT adrenal clock into a rhythmic output of corticosterone. WT hosts had normal rhythmic ACTH signaling input that was in phase with the adrenal clock and served to constructively propagate the rhythmic secretion of corticosterone to drive high-amplitude hormone release. Although this study was performed with mutant *Per2/Cry1* mice, it was concluded that mice carrying different clock gene mutations such as *Per1/2*-deficient mice were likely to produce similar results, although the sensitivity of the adrenal clock to ACTH may vary between mutants. Oster *et al.* (38) proposed a role for the peripheral adrenal clock as a local phase stabilizer for rhythmic peripheral hormone secretion.

The question of the function of the adrenal clock in relationship to the mechanism by which ACTH establishes the timed secretion of GCs has been investigated. Studies have shown that circadian rhythms of the steroidogenic protein StAR are absent in arrhythmic *Per1/2* and *Cry1/2* double-mutant mice. StAR is a target of ACTH and is the rate-limiting step in GC biosynthesis (39). This study was the first to establish StAR as an adrenal clock-controlled gene. GCs have long been thought to act as synchronizers of peripheral clocks (168, 169) by directly regulating the expression of *Per1* via distal GC-responsive promoter elements. Accordingly, the altered expression of *Per1* was attributed to the reduced levels of circulating GCs.

In summary, the adrenal peripheral clock is thought to have profound roles in harmonizing the mammalian circadian timing system, especially those slow-adjusting peripheral clocks, in response to changes in environmental stimulus via the rhythmic generation and secretion of GCs. In particular, *Per* plays an important role in modulating the circadian rhythm of GCs given the induction of *Per1* expression by nocturnal light exposure accompanied with subsequent secretion of corticosterone and loss of rhythmic secretion of ACTH as well as corticosterone in *Per1/2* double-mutant mice. Whether *Per1* mediates such synchronizing effects of GCs is yet to be further identified.

The endocrine pancreas

The endocrine pancreas is integral to glucose regulation *via* its secretion of insulin and glucagon (170). It is composed of a heterogeneous population of cell types. Pancreatic β- and α-cells secrete insulin and glucagon, respectively. Similar to other peripheral organs, endocrine pancreatic tissue exhibits a robust expression of major circadian clock genes (*Per1*, *Per2*, *Bmal1*, *Cry1*, and *Clock*) (171). Plasma levels of insulin and glucagon have also been show to cycle (172, 173), which is thought to be regulated by the pancreatic circadian oscillator (174–176), as illustrated in Fig. 3. Subsequent studies provided *bona fide* evidence for the role of the pancreatic circadian clock in regulating its function in rodents and humans (42, 177). β-Cell–specific *Bmal1* knockout mice exhibit phenotypes such as defective insulin production and severe glucose intolerance, indicative of β-cell dysfunction (175, 178, 179). This strongly supports a role for the β-cell intrinsic oscillator in insulin secretion and glucose homeostasis.

The effect of environmental factors (*e.g.*, nighttime light exposure) on the pancreas revealed novel insights on the role of *Per* genes in endocrine pancreas function. Although changes in the LD cycle *in vivo* entrain the phase of islet clock transcriptional oscillations, 10 weeks of continued exposure to light at night impairs the amplitude, phase, and inter-islet synchrony of clock transcriptional oscillations (180). Interestingly, it was observed that glucose regulates the amplitude and period of Per1 oscillations, indicating a nutrient-sensing mechanism in the islet clock. It has also been reported that constant light regimes or 6-hour advances of the light cycle every third day accelerate the development of diabetes in rats transgenic for the human islet amyloid polypeptide (181). Exposure to a high fat–containing diet has been shown to change the circadian expression pattern of Per1, and Per2 in mouse pancreatic islets, as well as circadian insulin secretion (182). Thus, environmental conditions that affect circadian rhythms can also impair the pancreatic clock and the function of this endocrine organ.

"Environmental conditions that affect circadian rhythms can also impair the pancreatic clock and the function of this endocrine organ."

Bone

There are cycling functions in both the developing bone, such as osteoblast proliferation, and in the adult bone during the remodeling process involving a continuous turnover of mineralized bone mass. Analysis of the bones of *Per1/2* double-knockout mice provided the first evidence for a role of clock genes in leptin-regulated bone metabolism. Specifically, *Per*-deficient mice show elevated bone mass and a paradoxical further bone mass increase following central leptin infusion. Thus, the circadian clock, and *Per* genes in particular, appear to mediate leptin-dependent sympathetic regulation of osteoblast proliferation and bone formation (183). Additional phenotypes were later described in *Per2*- and *Cry2*-deficient mice (184), showing increased bone volume in femoral, tibial, and lumbar spine bones. Histological analyses of the *Per2* knockout mice revealed significantly more osteoblast proliferation and mineralization activity, whereas in *Cry2* knockout mice osteoclast activity is reduced. Alternatively, *Per2*/*Cry2* double-knockout mice have normal bone mineralization when compared with the WT littermate controls at the age of 12 weeks. However, at a later stage (>1 year of age) these knockout mice suffer from osteoporosis. One should keep in mind that with global knockout mice the registered phenotype is a sum of many effects that are either directly or indirectly involved in bone morphology (185). More recently, clock genes have been found to influence bone development, density, and morphology, with the key findings reviewed elsewhere (185).

Adipose tissue

Adipose tissue is involved in energy homeostasis *via* the secretion of various bioactive substances known as adipo(cyto)kines (186). Clock genes (*Bmal1*, *Per1*, *Per2*, *Per3* *Cry1*, *Cry2*, and *Dbp*), adipokines (adiponectin, resistin, and visfatin), and genes encoding enzymes required for lipid metabolism (fatty acid transport protein 1 [*Fatp1*], acetyl-coenzyme A synthetase 1 [*Acs1*], and adipocyte differentiation-related protein [*Adrp*]) exhibit robust circadian rhythms. Thus, a functional circadian clock machinery in adipocytes may be involved in maintaining lipid metabolism (187, 188).

In an analysis of preadipocyte differentiation into mature white adipocytes, primary cultures of preadipocytes exert circadian oscillations of *Per2* and *Per3* transcripts, but not *Per1* (189). Such variations in circadian regulation of specific *Per* genes have been reported for a number of different tissues (137), suggesting tissue-specific functions of the three *Per* homologs in clock regulation and the coordination of rhythmic clock outputs. The high-amplitude activity profile of *Per3* in adipocytes (and adipose stromal vascular cells) suggests an important role for this gene in adipose tissue regulation (190), contrasting its rather negligible role in SCN pacemaker function (26). Consistently, when *Per3* is suppressed by small interfering RNA, adipocyte precursors differentiate faster and more completely into mature adipocytes, suggesting a specific role for *Per3* in fat tissue differentiation and lipid storage. Interestingly, PER3 interacts with KLF15 in adipocytes and might in this way contribute to lipid handling as an adaptation to nutrient availability (190).

PERIOD and the Regulation of Endocrine Signaling

Pineal melatonin and metabolism

The central pacemaker coordinates a broad range of downstream outputs of both neural and hormonal nature (116, 191, 192). SCN neurons exhibit robust rhythmic neuronal firing which peaks during the day and regulate activity rhythms in neighboring hypothalamic structures. Arginine vasopressin is one of neurotransmitters found in the SCN, which can regulate not only circadian rhythmicity of locomotor activity but also hormone release. Often neuronal and hormonal outputs are combined to propagate temporal information throughout the body. For example, indirect projections from the SCN target the pineal to regulate melatonin biosynthesis and release (116). In rodents, melatonin communicates with the pars tuberalis of the hypothalamus, a brain structure responsible for transducing temporal signals in the form of hormonal signatures to target tissues in the periphery (117). It has been shown that removal of the pineal abolishes *Per1* expression in the pars tuberalis, indicating that melatonin also plays an important role in remotely driving oscillatory clock gene expression. This is likely an understatement for the role of melatonin considering that PER proteins provide time-delayed inhibition of the transcription factor BMAL1, contributing in tissue- and organ-specific gene expression patterns and chromatin modifications that oscillate within a ~24-hour period.

Plasma melatonin concentration follows a daily rhythm with high levels during the night in all vertebrates [reviewed in Ref. (193)]. Thus, melatonin provides an important rhythmic endocrine signal for darkness in the body. Whereas the rhythmic synthesis and release of pineal melatonin is regulated by the circadian system, the location of the oscillator driving this rhythm is species specific (193, 194). In mammals, rhythmic melatonin synthesis in the pineal is controlled by the master clock in the SCN (195). The SCN drives nocturnal melatonin synthesis via the sympathetic nervous system (196). The nocturnally released sympathetic neurotransmitter norepinephrine (NE) activates β_1-adrenergic receptors in the pinealocyte membrane. This results in an increase in intracellular cAMP levels, signaling to CREB activation

(phosphorylation) and the subsequent transcriptional activation of genes with cAMP response elements (*CRE*s) in their promoters such as the gene encoding for the penultimate enzyme of melatonin synthesis, arylalkylamine *N*-acetyltransferase (*Aanat*) (197–200), and the clock gene *Per1* (201). In rodents, *Aanat* mRNA is rhythmically expressed with an impressive ~150-fold increase in concentration during late night (198, 202). Whereas the SCN clock is driving pineal melatonin synthesis in mammals, the pineal also houses its own circadian oscillator capable of maintaining rhythmicity *in vitro* (36, 203–205). Despite that the rat *Aanat* promotor region comprises an E-box element that can be activated by oscillating BMAL1: CLOCK heterodimers (206), pineal *Aanat* mRNA expression remains arrhythmic *in vitro* (206). Considering that the responsiveness of the pineal to NE is daytime-dependent, it was suggested that the pineal oscillator functions in gating (fine tuning) the timing of melatonin synthesis instead of driving it (207). Still, the mechanisms of gating in the regulation of *Aanat* gene expression in the pineal are not yet fully elucidated. However, it is speculated that the fine tuning of the neuroendocrine signaling in the pineal gland is dependent on a variety of neurotransmitters and neuromodulators, as well as on translational and posttranslational mechanisms. The different inputs on pinealocytes affect the shape of the melatonin signal by interacting at various levels with the NE/cAMP, pCREB/inducible cAMP early repressor pathway (208–213).

What significance does a gating mechanism have in the regulation of pineal melatonin synthesis? The key is in defining the term "nocturnal." Defining melatonin as the "hormone of darkness" or "nocturnal melatonin" implies that melatonin is synthesized only during the nighttime. This, however, is not a very accurate description of the temporal profile of nighttime melatonin. Melatonin has a species-specific nocturnal profile (193, 194, 214) determined by the onset of melatonin synthesis, its rate of synthesis, the timing and length of nighttime peak melatonin levels, and the offset of melatonin synthesis. Thus, the gating may serve a structural role in determining the shape of the nocturnal melatonin profile. One mechanism by which the pineal oscillator could gate the nocturnal profile is through a secondary role of clock genes. The clock gene *Per1* particularly among other clockwork components has been shown to cycle rhythmically in the pineal gland (193, 215), which persists *in vitro* (207). As shown in other brain areas, including the SCN and hippocampus, and in the periphery, cycling *Per1* may impose structure to the nighttime melatonin profile by modulating key signaling events involved in melatonin synthesis. It has been postulated for many years that the PER1 protein may act as an indirect inhibitor of its own transcription, likewise to the described feedback loop in *Drosophila* (216, 217). A possible mechanism for this autoregulation in mammals is a PER1-dependent modulation in the activity of its own transcription factor, CREB (77), which would suggest a regulatory function on melatonin synthesis. As described earlier, pCREB is important for *Aanat* expression, because similar to the promotor region of the *Per1* gene, it also contains *CRE*s (218). A study by Christ *et al.* (214) addressed this question by analyzing the temporal profiles of *Aanat* mRNA, pineal melatonin, AANAT enzyme activity, and plasma melatonin concentrations among other rhythms in melatonin-proficient mice either expressing or deficient for *Per1*. The results confirm that *Per1* increases the amplitude of *Aanat* expression. Hence, the clock protein PER1 plays an important role in the modulation of rhythmic melatonin synthesis in the pineal gland. As such, *Per1*—indirectly by structuring the nocturnal melatonin signal—is involved in regulating a plethora of central and peripheral melatonin-dependent physiological functions (219, 220).

Both *Per1* and *Per2* are expressed in a circadian fashion within the pineal glands of rodents (rats and mice) controlled by sympathetic afferent innervation (221). However, unlike *Per1* and *Aanat* expression being regulated by β-adrenergic signaling, *Per2* expression involves an alternative sympathetic route (208, 221). This also is supported by the observation that *Per2* transcription increases before the LD transition whereas *Per1* mRNA, in parallel to the accumulation of *Aanat* mRNA transcripts, rises at the onset of the night (208, 217, 221, 222).

"This suggests that peripheral clocks may be entrained independently of the central pacemaker."

PERIOD and the food-entrainable oscillator

A number of studies have shown that besides light, other cues, especially food intake, have substantial effects on the behavior and physiology of an organism (129, 223–225) (Fig. 4). A subset of clocks whose phase can be reset by the time of food availability to coordinate activity behavior and, consequently, food intake is collectively known as the food-entrainable oscillator (FEO). The FEO is considered a "black box" mystery in that its locus within the body is still unknown, and even the involvement of canonical clock genes is a matter of debate. A number of candidate structures have been proposed to harbor FEOs such as ghrelin-secreting cells in the stomach and a variety of extra-SCN nuclei within the brain, including the dorsomedial and lateral hypothalamic nuclei, to name a few [for a review, see Ref. (226)]. The current consensus is that the FEO consists of a multioscillatory system distributed throughout the body (227).

Damiola *et al.* (225) showed that applying restricted feeding schedules to animals under either LD or DD conditions changes the phase of circadian gene expression in peripheral tissues such as the liver, kidney, heart, and pancreas by up to 12 hours, whereas the phase of clock gene expression in the SCN remains phase-locked to the LD cycle. Food-induced phase

resetting of peripheral clocks coincides with the development of food-anticipatory activity (FAA) toward the end of the fasting phase. In *ad libitum* feeding conditions, however, FAA is not present and the phase of clock gene expression in peripheral tissues is coupled to that in the central pacemaker in the SCN. Such findings were confirmed by Stokkan *et al.* (139), who found similar decoupling of central and peripheral clocks. Taken together, this suggests that peripheral clocks may be entrained independently of the central pacemaker. Furthermore, light is the dominant *Zeitgeber* for most circadian clocks except in conditions where food is temporally restricted. As detailed in Fig. 4, under such circumstances, food availability exerts its dominant *Zeitgeber* influence on the phase of peripheral clocks, which is understandable in the context of food acquisition as the primary objective for most mammalian species, as it is necessary for survival. These findings are consistent with a recent report in humans, showing that altered meal timing can shift plasma glucose rhythms (129). The study shows that *Per2* expression shifts with meal timing, although to a smaller change compared with plasma glucose levels; nonetheless, the data indicate that feeding patterns may be capable of synchronizing peripheral clocks known to contribute to glucose homeostasis and less toward lipid metabolism. This confirms that timed meals in humans (129, 228, 229), as is the case in rodents (42, 175, 179, 230–232), may synchronize peripheral circadian rhythms, which has particular relevance for patients with circadian rhythm disorders, shift workers, and frequent transmeridian travelers.

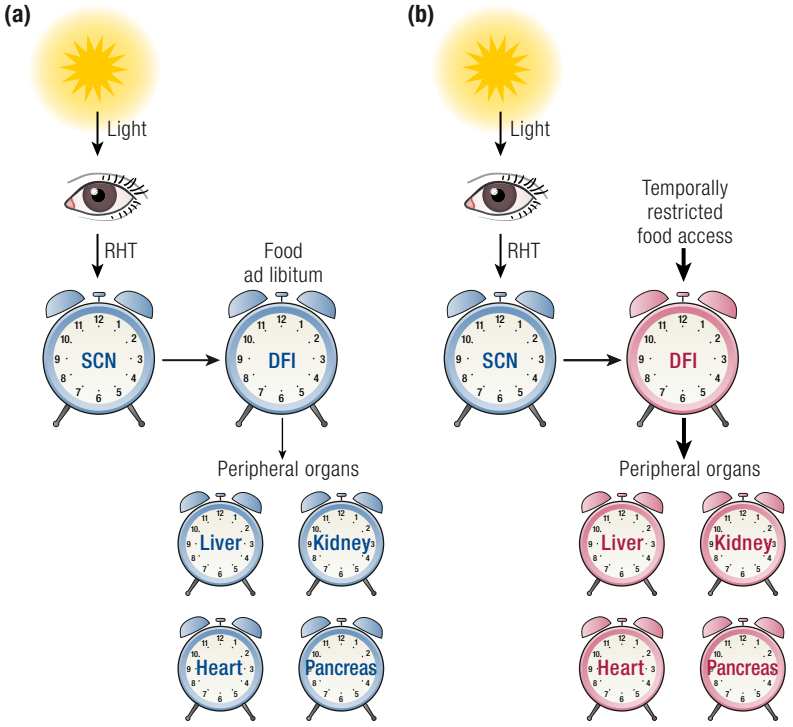


Figure 4. Hypothetical schematic depicting the hierarchical organization of the circadian network. (a) When food is available *ad libitum*, the photic input is the dominant time cue or *Zeitgeber*, and the light-entrainable oscillator (LEO) in the SCN functions as the master regulator of peripheral circadian clocks. The coupling of the SCN to peripheral oscillators is mediated via humoral and neuronal pathways. (b) However, under conditions of restricted food access, daily food intake (DFI) becomes the dominant *Zeitgeber* and the FEO is now the central clock system to drive and synchronize peripheral oscillators (bold arrows). Adapted under a Creative Commons Attribution 4.0 international license from Damiola F, Minh NL, Preitner N, *et al.* Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in suprachiasmatic nucleus. Genes and Development 2000;14:2950–2961 (225). [© 2019 Illustration Presentation ENDOCRINE SOCIETY].

Studies investigating the role of mammalian *Per* genes in FEOs have generated conflicting results. Feillet *et al.* (233) found that, although WT and *Per1* mutant mice express regular FAA, anticipatory behavior is abolished in $Per2^{Brdm1}$ mutant mice. Given previous investigations showing that mice mutant for *Clock* also showed normal FAA, Feillet *et al.* concluded that the contribution of different core clock gene components in the regulation of entrainment to food is not equal and that *Per2* may have a specific role in initiating FAA in mammals. Such findings were contested later by Storch and Weitz (234), who demonstrated that *Bmal1* knockout mutant mice lacking functional circadian clocks in all tissues showed robust FAA. Single-mutant *Per2*-null mice as well as double *Per1*/*Per2* knockout mice similarly displayed robust, stable, and quantitatively normal FAA. It was suggested that FEOs may rely on a yet unknown oscillatory system. The discrepancies are likely due to differences in the experimental protocols, specifically, in the time of day that mice were given access to food, which differed by 5 hours across both studies. This may have led to differences in energy homeostasis in mice of both studies.

Clock genes are largely expressed in areas of the mesolimbic system relevant for the hedonic and reward-associated control of appetite and food-seeking behavior. For example, *Per1–3*, *Clock*, and *Npas2* exert circadian oscillations in nuclei such as the accumbens, prefrontal cortex, amygdala, or bed nucleus of the stria terminalis (235). This appears also to be true for the lateral habenula, where PER2 shows an oscillatory behavior, possibly to tonically control dopamine release within mesolimbic areas (236). The diurnal and circadian function of the mesolimbic reward system may therefore be under the control of several external stimuli, such as food availability and intake (237). Anticipation of palatable food alters the expression of *Per1*, with increases in the nucleus accumbens and the prefrontal cortex (238). This interaction of food intake, anticipation, and rhythmicity of clock gene expression in hedonic brain areas is clearly a theme of greatest relevance for the understanding of (mis)timed and altered eating behavior.

Mendoza *et al.* (239) reassessed the effects of mutations in core clock genes on FAA in mice and found that single mutations of *Per1* and double

Per1/Per2-deficient mice displayed FAA expression that was significantly blunted compared with WT mice. Incongruently, they also found that $Per2^{Brdm1}$ mice did not exhibit FAA. Storch and Weitz (234) proposed the discrepancies to be linked to the different types of mutations used to knockout *Per2*. Both Feillet *et al.* (233) and Mendoza *et al.* (239) used $Per2^{Brdm1}$ mice whereas Storch and Weitz (234) used mice homozygous for a targeted disruption of *Per2*, referred to as $Per2^{-/-}$. Accordingly, it was suggested that the $Per2^{Brdm1}$ mutation may not act as a pure loss-of-function allele. Therefore, repetition of Feillet *et al.*'s and Mendoza *et al.*'s studies using $Per2^{-/-}$ mutant animals may warrant further investigation to confirm the role of *Per* genes in mammalian FEOs as proposed by Storch and Weitz (234).

An alternative theory of *Per* function in mammalian FEOs was proposed by Oster *et al.* (167) titled the "hierarchy of activity potentials" hypothesis. The hypothesis states that a hierarchy exists within the negative limb of the core TTFL (which consists of PER1, PER2, CRY1, and CRY2) to repress BMAL1 activity (Fig. 1). This hypothesis was founded on the premise that circadian arrhythmicity in mammals was largely due to irregularities in BMAL1 activity that occur secondary to disruptions in these proteins. Based on their observations, the authors concluded that CRY1 is a stronger repressor of BMAL1 than CRY2 whereas PER2 is more potent than PER1. Within the TTFL, a multimeric complex composed of at least two CRY proteins and two PER proteins forms in the cytoplasm and eventually translocates to the nucleus to repress BMAL1. Oster *et al.* suggested that the total activity potential (TAP) of this complex is dependent on the summation of individual potentials of the proteins, and that this TAP is ultimately responsible for whether BMAL1's transactivatory activity is affected by mutations in its components. For example, the standard WT PER–CRY complex is composed of CRY1, CRY2, PER1, and PER2, which has an intermediate repressor potential for BMAL1 and thus maintains a normal TTFL cycle. However, arrhythmicity results in mice that have complexes where the TAP is too high or too low. For instance, in $Per2^{Brdm1}$ mice, the PER–CRY complex consists of only PER1 proteins, and both paralogs in CRY1 result in a lower TAP compared with WT, which may account for the observed arrhythmicity and lack of FAA in these mice. Alternatively, in $Per2^{Brdm1}/Cry2^{-/-}$ mice the PER–CRY complex contains only *Per1* and *Cry1*, resulting in an overall intermediate TAP that may then account for the rescued rhythmicity and FAA in these animals. Recent studies have indicated that *Bmal1* and *Clock* are principally dispensable in FAA (234, 240). Thus, it is possible that this hypothesis may have to be adapted further to fit the current FEO model.

It is well known that food intake in organisms not only functions as a synchronizing cue but also contributes to the total energy homeostasis of the body. Energy homeostasis describes the state in which an animal's energy (food) intake is equal to its energy expenditure. This balance is maintained by molecular metabolic processes that occur in peripheral organs such as the liver and kidney. These processes may be driven or subdued by circulating hormones produced by endocrine glands to allow the organism to use its energy stores in situations when it is most needed (*e.g.*, intense physical activity, fasting, and stress).

Within the field of chronobiology there is clear consensus that molecular mechanisms of circadian oscillators and the regulation of metabolic homeostasis are intimately linked [reviewed in Ref. (241)]. Metabolic disorders such as obesity and diabetes are becoming more prevalent worldwide (242, 243). Although such diseases may in part be attributed to genetics and hyperalimentation, environmental factors such as the urbanization of developed countries, sedentary lifestyle, and increasing numbers of night shift workers are important contributors to the rising incidence and prevalence of metabolic diseases. The exposure to conditions that promote disengagement from natural circadian rhythms in an attempt to accommodate work and social demands (244) leads to "circadian misalignment," a term comprising desynchrony between internal circadian rhythms and the 24-hour solar cycle, that is, inappropriate timing of sleep and wake phases, as well as dissociation of central and peripheral rhythms (245). Circadian misalignment can cause adverse effects on health, as circadian clocks have been implicated in a plethora of physiological functions, including cardiovascular activity, renal function, endocrine systems, and metabolism (246, 247). In fact, the relationship between the clock and metabolism is bidirectional, in which the circadian clock also constantly receives metabolic feedback. To acutely visualize how deep the roots of the circadian system penetrate physiological processes, particularly metabolism, requires a momentary disruption of the circadian system.

PERIOD and the Clock in Health and Disease

Mounting evidence suggests that the alignment of clocks throughout the body is essential for circadian function. This information is largely based on data from laboratory rodent studies where access to all tissue types at any developmental stage is possible, providing valuable insights into the dynamics and phasing of clock genes and clock-controlled genes between different tissues. In humans, however, the demonstration of the molecular basis of circadian control presents many unique challenges, including the lack of control of environmental and genetic variables and, of course, the difficulty in collecting biological samples. Multi-tissue, multispecies

meta-analyses are valuable approaches to provide further understanding of the human circadian system. Comparison between diurnal (human) and nocturnal (mice) mammals (Tables 1 and 2) reveals a conservation of the clock's molecular components and its dynamics. What differs between species is not the clock itself but the alignment of the clock in various organs with each other and with the external environment (*i.e.*, the relative phasing of gene expression). The clock in the SCN, however, is unique in that its responsiveness to the LD cycle shows the same circadian phasing in diurnal and nocturnal species. Furthermore, humans and laboratory rodents (diurnal and nocturnal species) show the same (highly characteristic) staggered phase relationship of *Period* gene expression in the SCN, with *Per1* peaking soon after sunrise, *Per3* peaking during midday, and *Per2* peaking in the afternoon (125, 44, 248, 249). This, however, has not been observed in extra-SCN brain regions or peripheral tissues. Interestingly, although for extra-SCN brain tissue one may expect that diurnal and nocturnal mammals exhibit antiphasic relationships concerning clock genes and clock-regulated genes, data by Li *et al.* (125) do not support such a view. The study shows that human/mouse differences in the phasing of circadian genes are at ~6.5 hours when comparing the peak times for genes reported as rhythmic in the mouse brain (250) with human data (125). In most peripheral tissues, however, the phase difference in the expression of clock gene orthologs is ~12 hours between diurnal and nocturnal species (251). It is critical to remember that most, if not all, dynamics in clock genes and clock-controlled genes of human tissues reported thus far likely reflect the product of the modern environment phasing these dynamics. Caffeine, social jetlag, nighttime artificial light exposure, and decreased daytime light exposure are all features of the modern environment that may negatively affect circadian clocks and rhythms (251–255).

Next, we provide insight on *Period* and the circadian clock in human disorders, complemented with rodent data to further highlight the translational aspect of laboratory findings.

Sleep disorders

Sleep is a regular state of natural rest observed in all mammals, birds, and fish. Although characterized by a reduction in voluntary body movement and decreased awareness of the surroundings, sleep is a highly active central nervous process modulating neurocognitive, metabolic, and immunological processes. The regulation of sleep comprises two tightly interacting processes, that is, a homeostatic as well as a circadian process (256). Sleep- as well as wake-promoting signals are integrated mainly at the level of the hypothalamus and further modulated by oscillating projections from the SCN to the ventrolateral preoptic nucleus (257). Changes in sleep pattern—for example, shortened, fragmented, or insufficient sleep—disrupts the rhythmic transcriptome within different organ systems. However, the link between sleep and the circadian system is bidirectional because alterations in circadian rhythmicity also modulate duration and quality of sleep. In our 24/7 Western societies, sleep disorders are increasingly common with consequent effects on physical and mental health, quality of life, and daytime performance. According to the 2005 Sleep in America poll of the National Sleep Foundation, 75% of adult participants reported suffering from sleep problems (258). The third edition of the International Classification of Sleep Disorders (ICSD-3) classifies circadian rhythm sleep–wake disorders (CRSWDs) as one of six major categories of sleep disorders. In general, CRSWDs are defined as chronic or intermittent impairments of physiological sleep–wake cycles. Several subtypes of CRSWD can be differentiated as advances *vs* delays in physiological sleep phase, or a completely uncoupled free-running subtype. CRSWDs are caused by impairment of the circadian clock system due to extrinsic misalignment by jet lag during travel over time zones or shift work, and intrinsic circadian rhythm sleep–wake disorders due to genuine disturbance of the circadian clock network.

When investigating the impact of clock genes on human sleep, naturally occurring polymorphisms in these genes are assessed in the context of pathological sleep phenotypes. Multiple polymorphisms in core circadian clock genes as *CLOCK*, *NPAS2*, and particularly in all three *PER* genes have been shown to be linked with clinical relevant sleep disorders as well as individual sleep timing and morning or evening chronotype (259–263). A recent genome-wide association study (GWAS) in almost 90,000 participants identified 15 significant loci with relevant impact on sleep habits. Seven of these loci directly affected core circadian genes, including *PER2* (264). Furthermore, distinct missense mutations in the *PER2* and *CKIδ* gene have been identified in patients with familial advanced sleep phase syndrome, an autosomal-dominant CRSWD (265, 266).

The effect of sleep deprivation on clock gene expression and promoter methylation in skeletal muscle and subcutaneous adipose tissue has been evaluated by Cedernaes *et al.* (267). In a crossover study of healthy men, sleep deprivation increased methylation in two promoter-interacting enhancer regions of *Per1* in adipose, although *Per1* mRNA expression did not change in either tissue. *Cry1* and *Bmal1* mRNA expression in skeletal muscle were both downregulated by sleep deprivation, whereas postprandial serum glucose was higher. The study concluded that acute sleep deprivation may disrupt the regulation of key circadian genes in metabolic tissues. In a further study where the duration of sleep disturbance was extended

to 1 week, the circadian mRNA expression of *Per1*, *Per2*, and *Per3* in whole-blood RNA samples was examined. Whereas all three *Per* genes remained rhythmic, *Per2* was upregulated during sleep deprivation and after sleep restriction, together with genes associated with the inflammatory response (268).

Psychiatric disorders

Beyond their impact on physical health and disease, circadian clock genes might also play a role in mental state and psychiatric disorders (269). Because sleep disorders are highly prevalent in psychiatric disease, there seems to be a tridirectional relationship connecting circadian rhythms, sleep, and mental health. One hypothesis for the development of psychiatric disorders related to impairment of the circadian network postulates an individual's reduced ability to adapt to environmental changes due to a mismatch between individual internal and external rhythms. Likewise, studies on early development of social communication highlight the necessity of environment-adapted synchrony in motor, emotional, and interpersonal rhythms for mental health (270). The early phase of physiological brain development is, among other factors, under control of clock genes (271). Therefore, an impaired clock network might contribute to a higher susceptibility for a later development of psychiatric disorders (272). A mechanistic link might be a disturbance in sensory processing by parvalbumin neurons under conditions of an insufficient circadian clock network. Similarly, a knockdown of parvalbumin in mice leads to an autism-like phenotype (273).

In humans, target gene approaches and GWASs have identified a possible link between single-nucleotide polymorphisms in relevant genes of the clock machinery and multiple psychiatric disorders. In patients with bipolar disorders, several polymorphisms in core clock genes have been associated with rapid cycling between episodes of mania and depression (*CRY2*) as well as the risk of bipolar disorder and schizophrenia onset (*PER3*) (274, 275). Moreover, a recent study showed an association between weaker cyclic patterns in *PER1–3* and *BMAL1* in brains of patients with major depression as compared with healthy controls (125). Similar associations have been shown for the psychiatric spectrum of attention deficit hyperactivity (276) and anxiety disorder (277).

Human studies have relied on associations that are unable to establish causality. Impairment of circadian rhythmicity is associated with sleep disturbances and psychiatric disorders, but we cannot conclude which is the chicken or the egg. One possible mechanistic link between impairment of the circadian clock network, sleep disorders, and psychiatric disorders might be alterations in GC rhythms, which are highly relevant in the context of mental health as well as sleep (278, 279).

Obesity

Existing evidence points to a strong genetic association between the circadian system and obesity. A GWAS exploring the genetic predisposition of obesity with hypothalamic genes disclosed that common variants in *PER1* and *NUCB2*, a gene coding for nesfatin-1, an adipokine involved in the circadian regulation of food intake, show a significant allelic association with obesity (280). Accordingly, it might be postulated that circadian disruptions lead to metabolic diseases. Indeed, circadian alterations caused by shift work and sleep deprivation have been linked to unfavorable metabolic sequelae [reviewed in Ref. (281)]. Shift workers suffering from disrupted circadian physiology and sleep disorder are more prone to eat at night and become obese (282, 283). Even short exposure of normal, healthy, young adults to sleep deprivation in the laboratory increases appetite and food intake associated with decreased leptin and increased ghrelin levels (284, 285), reflecting a condition known as "night eating syndrome." Animal research reveals a direct link between circadian clock genes and night eating syndrome. A study in mice deficient in the clock protein PER2 shows that the circadian clock regulates two critical metabolic rhythms, GC release and food intake (165), both of which are absent in $Per2^{-/-}$ mice. On a high-fat diet, the food intake in $Per2^{-/-}$ mice is similar across the light and dark phases and results in significant obesity. The diurnal rhythm of neuroendocrine peptide α-melanocyte-stimulating hormone (αMSH), a major effector of appetite control, is disrupted in $Per2^{-/-}$ mice. Peripheral injection of αMSH, which has been shown to enter the brain, restores the feeding rhythm and induces weight loss in $Per2^{-/-}$ mice. These findings emphasize the importance of *Per2* in appetite control during the sleeping phase and the potential role of peripherally administered αMSH in restoring a night–day eating pattern in individuals with circadian eating disorders such as night eating syndrome, associated with obesity. Additional studies in mice also demonstrated that animals with access to a high-fat diet restricted to the sleep phase for 6 weeks gain 2.5-fold more weight than do control mice fed during the active phase (286). These findings are further supported by a recent study (287) showing that 1 week of restricted feeding during the inactive phase causes hyperphagia and increasing adiposity and plasma leptin levels as compared with nighttime feeding. Mistimed food consumption also prominently affects the expression of circadian clock genes (*Bmal1*, *RevErbα*, *Per1*, and *Per2*), which show either phase advances or inverted expression profiles in liver and white adipose tissue in inactive phase mice. Moreover, time-restricted access to a high-fat diet during an extended period (18 weeks) ameliorates the disruptive effects of diet-induced obesity. Mice fed with a high-fat diet during the active phase gained 18% less body weight, decreased serum cholesterol by 30%,

"The timing of feeding has a pivotal role and can ameliorate the detrimental effects of an obesogenic diet."

and increased insulin sensitivity more than threefold compared with mice fed *ad libitum*. More importantly, the disrupted circadian expression profiles of the clock genes *Per1*, *Cry1*, and *Clock* in the liver induced by an *ad libitum* high-fat diet can be restored via a time-restricted high-fat diet (288). As such, the timing of feeding has a pivotal role and can ameliorate the detrimental effects of an obesogenic diet.

In humans, both the expression levels and circadian rhythmicity of clock genes, including *Per1*, *Per2*, and *Per3*, in subcutaneous adipose tissue are similar in lean, overweight/obese, and overweight/obese individuals with type 2 diabetes mellitus (T2DM) (133). Nevertheless, there appears to be good evidence that the expression of clock genes is altered by weight changes. As such, *PER1* and *NR1D1* expression is increased in subcutaneous adipose tissue after a period of 8 weeks of weight loss. Expression levels are tightly correlated with body mass index (BMI), serum cholesterol levels, and several metabolic and inflammatory genes. These data also emphasize the close interaction between clock changes and alterations in adipose tissue mass and metabolism (289).

The adipokine leptin, a potent appetite suppressant, plays an important role in the interaction of peripheral signals with the SCN to maintain energy homeostasis. Plasma leptin levels show robust diurnal rhythms in both humans and rodents that are closely associated with food intake. Food availability restricted to the inactive phase in mice reverses the diurnal leptin rhythm (290). Leptin resistance is a hallmark of obesity and is significantly affected by circadian disruptions such as jet lag or clock gene knockout in mice (291). Kettner *et al.* (291) demonstrated that both acute and chronic jet lag alone is sufficient to induce leptin resistance by disrupting cycling leptin concentrations. The importance of circadian clocks in leptin regulation was further emphasized by findings showing that *Per1/2* double-knockout mice display abolished daily variations in plasma leptin resulting in complete loss of leptin feedback to the arcuate nucleus. These findings suggest that food intake at the wrong time of day can desynchronize peripheral clocks, resulting in metabolic disorders by mechanisms involving leptin resistance, hyperphagia, physical inactivity, and adiposity (Fig. 5).

The question as to which of these physiological changes that may contribute to obesity are actually mediated by the circadian clock, has been raised by Husse *et al.* (292). This study investigated the role of the circadian clock machinery in metabolism under conditions of sleep disruption using the mouse model. The group examined the effects of timed sleep restriction (TSR)—a protocol that sleep deprives mice for their first 6 hours of sleep time during the light phase—in WT and in arrhythmic *Per1/2* double-mutant mice. WT mice subjected to TSR show increased food intake, hyperleptinemia, and a significant upregulation of genes involved in lipid biosynthesis in white adipose tissue. These changes persist for at least 1 week after TSR and thus indicate the onset of obesity and associated leptin resistance. Importantly, TSR-induced persistent metabolic effects observed in WT mice were absent in *Per1/2* mutants, suggesting a protective role of *Per* genes on metabolic deterioration resulting from sleep disruption (292). The extent to which these protecting effects are mediated by *Per1* or *Per2* or the overall disruption of endogenous rhythmicity remains to be determined.

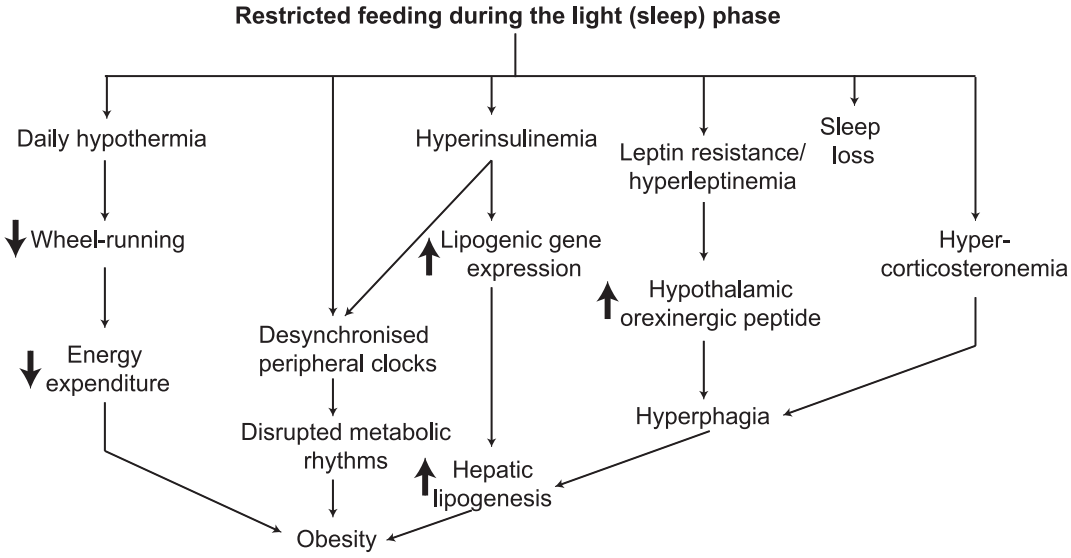
Type 2 diabetes

The epidemic of diabetes is increasing at an alarming rate, concurrent with the prevalence of obesity throughout the world. T2DM is a multifactorial complex metabolic disease that occurs due to an interaction between genetic predisposition and environmental factors (14, 181). A number of studies support the involvement of circadian clock genes in regulating glucose metabolism and diabetes. Clinical studies in individuals suffering from sleep disruption or circadian misalignment show an increased susceptibility and incidence of T2DM, evidenced by insulin resistance, impaired glucose tolerance, and hyperglycemia (293–296). A study by Englund *et al.* (297) addressed whether circadian clock polymorphisms contribute to seasonal variations in behavior and to the metabolic syndrome in humans. The group genotyped 39 single nucleotide polymorphisms from 19 genes that were either canonical circadian clock genes or genes related to the circadian clockwork from 517 individuals. The findings link circadian gene variants to the risk factors of the metabolic syndrome, because *Npas2* was associated with hypertension and *Per2* was associated with high fasting blood glucose.

Mice deficient for the core clock genes *Bmal1* or *Clock* display impaired recovery of normal blood glucose levels after insulin-induced hypoglycemia, indicative of a dysfunction in hepatic gluconeogenesis (298). Furthermore, prominent hepatic sequelae of the metabolic syndrome such as steatosis, hyperlipidemia, and altered secretion of hepatokines are observed in these mice (299), stressing the importance of the circadian clock in glucose homeostasis.

Rodent studies show that the expression of clock genes is significantly affected in nonobese mice with insulin resistance, a contributor to the development of T2DM (300, 301). Furthermore, nonobese *Cry1* mutant mice show early onset diabetes mellitus with symptoms similar to those of maturity onset diabetes of the young in humans in terms of early onset, nonobesity, and primary dysfunction of β-cells (302). In accordance with these findings, administration of glucose has been shown to modify the expression of *Per1* and *Per2* in fibroblasts and immortalized hypothalamic cell lines (303, 304). Clinical studies have

Figure 5. Linking mistimed feeding to obesity in mice. The inappropriate timing of food consumption can desynchronize peripheral clocks and cause metabolic disorders by mechanisms involving leptin resistance, hyperphagia, physical inactivity, and adiposity, which are leading causes for obesity. Reproduced with permission from Yasumoto Y, Hashimoto C, Nakao R, *et al.* Short-term feeding at the wrong time is sufficient to desynchronize peripheral clocks and induce obesity with hyperphagia, physical inactivity, and metabolic disorders in mice. Metabolism 2016;65:714–727 (287).



also shown that the mRNA expression levels of core clock genes (*Clock*, *Bmal1*, *Per1*, *Per2*, *Per3*, and *Cry1*) were significantly lower, and amplitudes of mRNA expression rhythms tended to diminish in leukocytes of patients with diabetes compared with nondiabetic subjects (305).

The effect of skipping breakfast on clock gene expression in leukocytes was compared in people with and without T2DM (306). Omitting breakfast had a significant effect on subsequent mRNA expression levels of several clock genes. Compared with a day where the participant had breakfast, omitting breakfast results in lower afternoon (after lunch) expression of *Per1* in the diabetic group. *Per1* and *Per2* increase after lunch on the day when the participant has breakfast. Individuals with type 2 diabetes who omitted breakfast demonstrated a greater disruption to their circadian rhythms than did those who had breakfast.

A recent study by Qian *et al.* (307) showed, to the contrary, that in an animal model for T2DM, employing rats transgenic for human islet amyloid polypeptide overt diabetes did not modify circadian function or molecular rhythms in *Per1* expression. Because other studies in leptin-resistant and leptin-deficient mice (*db*/*db*, and *ob*/*ob*, respectively) have shown that these animals exhibit a reduced circadian activity (308), it appears plausible that obesity and associated biochemical phenotypes (*i.e.*, altered adipokine secretion) may mediate circadian disturbances observed in T2DM.

In the liver of insulin-resistant mice, levels of *Bmal1*, *Cry1*, and *Per2* mRNA expression were found to be significantly elevated at the ages of 16 to 32 weeks, whereas *Per1* mRNA expression was significantly elevated after the age of 32 weeks (301). Correspondingly, a subsequent study showed elevated clock gene expression (*Per1*, *Per2*, *Per3*, *Clock*, *Bmal1*, *Cry1*, and *Cry2*) in the liver of 32- and 48-week-old mice (300) that was lost in 60-week old mice, an effect that was associated with severe diabetes. Yang *et al.*'s (301) previous study showed persistence of the circadian rhythmicity in gene expression throughout the experimental time course, whereas a subsequent study by Tseng *et al.* (300) showed a loss of 24-hour circadian rhythms in gene expression, indicating that early alterations in gene expression may act as an alarm system for imminent physiological changes. These findings suggest that re-establishing high-amplitude circadian rhythmicity could improve or reverse the glucose imbalance resulting from insulin resistance, and may also delay or prevent the onset of diabetes (300). Therefore, enhancement or maintenance of the stability of the circadian clock system may provide a novel therapeutic approach to improve glucose metabolism.

Despite a paucity of publications exploring the role of *Per1* in glucose homeostasis, there is a growing body of evidence demonstrating the roles of the *Per* gene family in liver function. The liver plays a pivotal role in energy metabolism by maintaining glucose homeostasis through a tight regulation of catabolic and anabolic processing of glucose in response to the energy demands of the body (139). The existence of a peripheral liver clock has been identified by Damiola *et al.* (225) and Storch *et al.* (114) and it is thought that

hepatocyte clocks are involved in maintaining metabolic homeostasis by integrating external food cues to temporally tune the expression of genes that encode for metabolism-associated enzymes. It appears that PER1 in the liver may be involved in food entrainment of local clocks. Glucose was reported to be a key step in triggering the expression of clock genes by downregulating *Per1* and *Per2* mRNA in rat-1 fibroblasts (304). Behavioral studies in mice show that acute food intake downregulates *Per1* transcripts in the liver by inhibiting CREB signaling (309) while upregulating *Per2*, possibly through a postprandial rise in body temperature leading to an increase in heat shock protein production, which then bind to a *cis*-acting enhancer element in the *Per2* promoter (141). Resetting of the liver clock by feeding has been attributed to a number of food-associated pancreatic hormones such as insulin and glucagon. Recently, oxyntomodulin, an anorexigenic gut-derived hormone stimulated by food intake, has been proposed as a novel direct pathway by which food may reset liver clocks via the specific induction of *Per1* and *Per2* (310).

Per1 has also been investigated for its role in hepatic lipid metabolism and is linked to diabetes by suppressing insulin signaling. PER1 activates peroxisome proliferator-activated receptor (*Ppar*)α (311), a master lipid regulator involved in the modulation of hepatic fatty acid oxidation (312), and Bhlhe40 (*Dec1*) and Bhlhe41 (*Dec2*), which are thought to be repressors of adipogenesis. The noncanonical mechanism involved suggests that PER1 suppresses *Cry2*, which in turn leads to the suppression of *Clock/Bmal1*. The function of *Per1* in hepatic fatty acid metabolism was further confirmed in mice lacking *Per1*, which show reduced ethanol-induced triglyceride accumulation in liver, alleviating ethanol-induced hepatotoxicity. Overexpression of *Per1* reverses this phenotype, leading to an upregulation of genes related to lipogenesis such as *Ppar*γ, a nuclear receptor critical for adipogenesis, and downstream target genes (313). Similarly, *Per2* has been found to directly and specifically repress *Ppar*γ by blocking its recruitment to target promoters (314). Ablation of PER2 in mice is associated with increased activation of adipogenic genes and markers and a drastic decrease in total serum triacylglyceride (TAG) levels (314). This interaction between *Per2* and *Ppar*γ seems to be paralog-specific, as *Per1*, despite sharing a high degree of homology with *Per2*, does not show any involvement in adipogenesis. Collectively, this indicates a role for the *Per* gene family in maintaining body fat and serum TAG levels in mammals by regulating liver fatty acid metabolism and adipogenesis. In contrast, recent evidence by Adamovich *et al.* (315) suggests that diurnal oscillations in hepatic triglycerides and mRNA expression of TAG metabolism-associated enzymes can persist in *Per1/2* double-knockout mice. It is unclear whether this is due to functional redundancy within the *Per* family, as no studies have examined hepatic lipid metabolism in *Per1/2/3* triple-knockout animals.

GC disorders

Successful adaptation to the environment depends on an organism's ability to appropriately modulate its stress response (316). Central to this modulation are the adrenals, whose cortex and medulla release hormones in a time-of-day–dependent fashion, with GC secretion being the best studied in terms of its circadian effects (317). The loss of circadian secretion of cortisol is one of the most sensitive and specific diagnostic features of Cushing syndrome, and thus is recommended by the guidelines to evaluate the disease state. Adrenalectomy in combination with postoperative GC replacement, using hydrocortisone, is a common treatment of Cushing syndrome.

A human study investigated the acute effects of hydrocortisone administration on *Per* gene expression in whole blood. Hydrocortisone resulted in induction of *Per1* and *Per3* but there was no effect on *Per2* mRNA expression (318). The hydrocortisone-induced increase in *Per1* mRNA was partly suppressed by the GC receptor antagonist RU486. The doses of hydrocortisone result in serum cortisol concentrations similar to those observed during acute stress, implying that peripheral *Per1* and *Per3* may be temporarily upregulated during acute stress in humans. When the hydrocortisone administration was administered repeatedly, the acute effect on increasing *Per1* expression was confirmed; this experiment used peripheral blood mononuclear cells. Additionally, after 6 days of hydrocortisone administration (20 mg/d taken 10 hours after their scheduled time of waking), the phases of *Per2*, *Per3*, and *Bmal1* were shifted by between 9.5 and 11.5 hours, further supporting the notion that peripheral clocks are entrained by GCs in humans (319).

Cardiovascular diseases

Epidemiological data report that cardiovascular events such as myocardial infarction, sudden cardiac death, angina pectoris, ischemic and hemorrhagic stroke, and ventricular tachycardia exhibit circadian variation (320, 321). Hemodynamic parameters such as blood pressure and heart rate as well as gene and protein expression of known regulators of vascular physiology show circadian oscillations (322–324). That is, human endothelial vasodilatory activity decreases in the early morning and coronary artery tone increases in the morning, whereas blood pressure and heart rate increase in the early morning. Therefore, chronodisruption/circadian misalignment as commonly observed in night shift workers significantly

elevates the risk of cardiovascular morbidity and mortality, highlighting the potential importance of the circadian clock in the cardiovascular system (325).

The association between circadian clock function and cardiovascular regulation is bidirectional. Chronic circadian desynchronization by repeated 12-hour phase shifts of the LD cycle significantly compromises survival of cardiomyopathic hamsters, and altering the LD cycle length from 24 to 20 hours (10 hours light/10-hours dark) exacerbates the severity of disease in mice suffering from cardiac hypertrophy (326). Circadian disruption by deletion or mutation of core clock genes leads to malfunctions in vasculature. Mice with *Per2* mutation or *Bmal1* knockout show significantly impaired endothelial nitric oxide–mediated relaxations in response to stimulation with acetylcholine, ATP, or ionomycin, a substantial increase in collagen deposition in arteries, and a higher susceptibility to thrombosis in ligated vessels compared with WT (327). More importantly, diurnal variations in vessel relaxation are absent in clock gene mutant mice (328). Although numerous publications report on the importance of circadian system function in physiological homeostasis, studies focusing on the role of *Per1* in cardiovascular physiology are very limited. Young *et al.* (329) reported that the circadian expression of clock output genes known as the PAR transcription factors (rich in proline and acidic amino acid residues), *Dbp*, *Hlf*, and *Tef*, is significantly attenuated in pressure-overload hypertrophic heart tissue despite the retained circadian expression of core clock genes (*Bmal1*, *Clock*, *Per1*, *Per2*, *Per3*, *Cry1*, and *Cry2*). The effects of cardiovascular disorder on circadian rhythms are further elaborated in obese mice that carry a high risk for various metabolic diseases, including cardiovascular disorder. In a study by Nernpermpisooth *et al.* (330) obese mice show a loss of the daily rhythm and significantly suppressed expression of *Per1* as well as of clock output genes such as *Dbp* under disrupted circadian conditions and in DD, indicative of impaired clock performance. Notably, responsiveness of the microcirculation of these mice to acetylcholine is significantly reduced and expression of genes involved in vascular relaxation including *eNOS* and *GTPCH1* is also prominently suppressed, collectively indicative of impaired endothelium-dependent relaxation.

There appears to be a pathophysiologically relevant role of *Per1* in the development of hypertension, less so for *Per2*. In particular, *Per1* apparently affects the regulation of renal sodium reabsorption through altering the basal and aldosterone-induced control of the α-subunit of the epithelial sodium channel (ENaC) in collecting duct cells. This channel critically regulates blood volume and thereby blood pressure. As such, inhibition of *Per1* nuclear entry decreases αENaC expression and activity in mouse kidney cells (331). This study also revealed that mice lacking functional *Per1* exhibit significantly reduced blood pressure compared with WT animals. Whether the reduced ENaC activity in the absence of *Per1 in vivo* contributes to the blood pressure phenotype observed in *Per1* knockout mice still needs to be clarified. Alternatively, a recent study by Solocinski *et al.* (332) reported a different role of *Per1* in the pathogenesis of hypertension. The authors sought to examine the effects of a high-salt diet plus mineralocorticoid treatment on *Per1*-mediated blood pressure in mice. This high-salt diet/mineralocorticoid intervention significantly elevated mean arterial pressure in *Per1* knockout mice and resulted in absence of the expected blood pressure drop. Accordingly, it was concluded that lack of *Per1* impairs the ability of the mice to decrease their blood pressure during the inactive phase, hence precipitating the onset of adverse cardiovascular events (332). Another study performed in humans demonstrated significantly enhanced *PER1* mRNA expression in the renal medulla of hypertensive patients when compared with normotensive controls. Whether this increase is causal or compensatory is unclear (333).

The role of *Per2* in the development of high blood pressure is even less understood; there is one report that *Per2* mutant mice show a reduced diastolic blood pressure, a moderate increase in heart rate, and a reduced nocturnal dipping of blood pressure. The mechanism behind this phenotype remains to be clarified (328). Taken together, the circadian clock plays a crucial role in the maintenance of normal cardiovascular functions. However, further in-depth studies are required to refine our knowledge on the specific role of *Per1/2* in cardiovascular physiology.

Conclusion

In living organisms, the circadian clock tightly organizes the temporal coordination of biological processes in accordance with environmental stimuli and demands. Not only central, but also peripheral clocks are important in this regard, and misalignment between different nodes of the circadian clock network can predispose individuals to physiological complications and diseases. Clock genes, including *Per*s, are actively involved, both in central and peripheral tissues, in maintaining the integrity of physiological functions. Table 3 provides the summary on functions of *Per* orthologs in the brain and periphery. *Per1* as well as *Per2* play a pivotal role in circadian clock resetting, and tissue-specific clock functions have been intensively investigated in studies of metabolic diseases. Not only *Per* genes but also genes related to numerous

"Misalignment between different nodes of the circadian clock network can predispose individuals to physiological complications and diseases."

Table 3. The Functions of *PERIOD* in Central and Peripheral Tissues of the Mouse

	Tissue	Function: Tissue	Gene	Function: Gene		References
				Common	Specific	
Central	SCN	Central pacemaker of circadian system	*Per1*	Photic resetting	N/A	(45, 51, 52)
			Per2		Maintenance of circadian rhythm stability	(45, 46, 52)
			Per3	Possible extra-clock functions		(218)
	Hippocampus	Learning and memory	*Per1*	Modulation of hippocampal memory-relevant signaling molecules and spatial working memory		(74, 75, 104)
			Per2	Memory recall		(96)
Periphery	Adipose tissue	Fat storage and regulation of metabolic homeostasis via the secretion of adipocytokines	*Per1*	Rhythmic regulation of leptin levels	N/A	(133, 280, 289, 291, 292)
			Per2	Energy homeostasis	N/A	(133, 289, 291, 292)
			Per3	N/A	Modulation of adipogenesis and lipid handling	(190)
	Liver	Regulation of glucose homeostasis	*Per1*	Modulation of homeostatic hepatocytes turnover	Regulation of hepatic lipid metabolism	(309, 310, 313)
			Per2	Maintenance of hepatocyte clock function	Regulation of adipogenesis	(309, 310, 314)
				Resets the hepatic clock		
				Regulates insulin response		
	Adrenal gland	Regulation and synchronization of physiological functions in periphery	*Per1*	Mediates light-induced secretion of GCs via the autonomic nervous system Regulation of rhythmic secretion of GCs and StAR expression		(39, 166)
			Per2	Regulation of adrenal sensitivity to ACTH; regulation of circadian rhythms of ACTH, GCs, and local clock		(167)
	Heart and vasculature	Blood, oxygen and nutrients supply and removal of metabolic waste products	*Per1*	Regulation of blood pressure rhythm		(331, 332)
			Per2			
Other	FEO	Resetting of the behavior and physiology of an organism by the time of food availability	*Per1*	Role of *Per* genes in FEOs remains controversial	Synchronization of peripheral oscillators according to the timing of feeding when feeding is restricted	(233, 234, 239)
			Per2			

Per1–3 are widely expressed throughout the brain and the periphery. Centrally, PERIOD plays a major role in light-induced clock resetting. In the periphery, circadian oscillators are necessary for maintaining metabolic homeostasis by synchronizing the associated physiological processes with environmental rhythms.
Abbreviation: N/A, not available.

physiological processes, including sleep and energy metabolism, have been reported to be significantly affected under the circumstances of chronodisruption. Also, genetic or diet-induced disease conditions can alter *Per* gene expression, emphasizing the bi-directional connection between circadian clock and physiology. Improvements in entrainability to different lengths of LD cycles and metabolic functions in the absence of *Per1* indicate that *Per1* may play a crucial role in synchronization between central and peripheral clocks.

We acknowledge that the *in vivo* evidence supporting a role of PER in endocrine and metabolic functions is currently based on global *Per* knockout studies in rodents and observations in humans with single nucleotide polymorphisms in the *Per* gene. The substantial amount of evidence provided in this review for the role of *Period* in endocrine and metabolic regulation as well as disorders should drive the generation of cell-specific *Per* knockout models to prove the specific and highly relevant role of *Period* genes on the tissue-specific

functions discussed in this review. This cell-specific function was also confirmed by *in vitro* studies addressing the role of *Period* genes by downregulating *Per* expression or by its removal (*e.g.*, in cultures derived from *Per* knockout mice) (75, 214, 313, 314).

Clearly, future studies specifically targeting *Per*s are required to elaborate their specific central and peripheral roles, which may then provide novel means to ameliorate metabolic and other sequelae of chronodisruption.

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Correspondence and Reprint Requests: Oliver Rawashdeh, PhD, Chronobiology and Sleep Group, School of Biomedical Sciences, University of Queensland, Otto Hirschfeld Building 81, Room 522, St. Lucia, Queensland 4072, Australia. E-mail: o.rawashdeh@uq.edu.au.

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Abbreviations

Aanat, arylalkylamine *N*-acetyltransferase; BMAL1, brain and muscle Arnt-like protein-1; CLOCK, circadian locomotor output cycles kaput; CRE, cAMP response element; CREB, CRE binding protein; CRSWD, circadian rhythm sleep–wake disorders; Cry, cryptochrome; DD, constant darkness; ENaC, epithelial sodium channel; FAA, food-anticipatory activity; FEO, food-entrainable oscillator; GC, glucocorticoid; GWAS, genome-wide association study; HPA, hypothalamus–pituitary–adrenal; LAD, light at day; LD, light/dark; LTM, long-term memory; LTP, long-term potentiation; NE, norepinephrine; PAS, PER-ARNT-SIM; pCREB, phosphorylated CREB; Per, period; PKA, protein kinase A; Ppar, peroxisome proliferator-activated receptor; SCN, suprachiasmatic nucleus; StAR, steroidogenic acute regulatory; TAG, triacylglyceride; TAP, total activity potential; T2DM, type 2 diabetes mellitus; TTFL, transcriptional–translational feedback loop; TSR, timed sleep restriction; WT, wild-type; αMSH, α-melanocyte-stimulating hormone.